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**Robust Summaries
for
Tris(nonylphenyl) phosphite
CAS Number 26523-78-4**

USEPA HPV Challenge Program

Final Submission

November 15, 2006

Submitted by:

Phosphite Manufacturers Consortium (PMC)
1250 Connecticut Avenue, NW, Suite 700
Washington, DC 20036
Phone: (202) 419-1500
Fax: (202) 659-8037

1.1.0 Substance Identification

IUPAC Name: Phenol, nonyl-, phosphite (3:1)
Smiles Code: CCCCCCCCC1ccc(cc1)OP(Oc2cccc(c2)CCCCCCCC)Oc3ccc(cc3)CCCCCCCC
Mol. Formula: C45H69O3P
Mol. Weight: 689

1.1.1 General Substance Information

Substance type: organic
Physical status: liquid
Purity: = 93.9 - 100 % w/w
Colour: clear liquid

1.2 Synonyms and Tradenames

Alkanox TNPP	Polygard LC
Irgafos TNPP	TNPP
Irgastab CH 55	Tri(nonylphenyl)phosphite
Lowinox TNPP	Tris(monononylphenyl)phosphite
Naugard TNPP	Trisnonylphenylphosphit
Polygard	Weston 399
Polygard HR	Weston TNPP

1.3 Impurities

CAS-No: 25154-52-3
EC-No: 246-672-0
EINECS-Name: nonylphenol
Contents: < 5 - % w/w

CAS-No: 25154-52-3
EC-No: 246-672-0
EINECS-Name: nonylphenol
Contents: < 3 - % w/w

CAS-No: 108-95-2
EC-No: 203-632-7
EINECS-Name: phenol
Contents: < .1 - % w/w

CAS-No: 25417-08-7
EINECS-Name: di(nonylphenyl)phenylphosphite
Contents: = .05 - % w/w

CAS-No: 7782-50-5
EC-No: 231-959-5
EINECS-Name: chlorine
Contents: = .005 - % w/w

(10)

1.4 Additives

CAS-No: 122-20-3
EC-No: 204-528-4
EINECS-Name: 1,1',1''-nitritotripropan-2-ol
Contents: ca. .5 - 1 % w/w

1.7 Use Pattern

Remarks: TNPP is used in the manufacture of a variety of plastic and rubber products, including polyvinylchloride (PVC) film, rubber, polyolefins linear low density polyethylene (LLDPE), high density polyethylene (HDPE), rubber-modified polystyrene and other products. Additional information can be found in the latest EU environmental risk assessment (February 2006).

Type: type
Category: Use resulting in inclusion into or onto matrix

Type: industrial
Category: Polymers industry

Type: use
Category: Stabilizers

1.7.2 Methods of Manufacture

Orig. of Subst.: Synthesis

Remark: Alkanox TNPP is produced by reaction between nonylphenol and phosphorus trichloride in presence of organic catalyst.

1.11 Additional Remarks

Remark: DISPOSAL METHOD: by controlled incineration.
TRANSPORT INFORMATION: ONU number 3082
Rail/road(RID/ADR): class 9 11^ c
Sea(IMO/IMDG): Not Restricted
AIR(ICAO-IATA): Not Restricted

2.1 Melting Point

Value: = 6 degree C
Decomposition: no at degree C
Sublimation: no

Method: OECD Guide-line 102 "Melting Point/Melting Range"
Year: 2001
GLP: yes
Test substance: Tris-nonylphenyl phosphite (TNPP; CAS#26523-78-4; Lot #173T110700 from Dover Chemical Corporation): Purity: 99.8%

Remark: The pour point was determined for tris-nonylphenyl phosphite instead of the melting point. A melting point could not be observed using the differential scanning

calorimetric (DSC) method because an endothermic event was not observed in the heat flow versus temperature plot. This was probably due to the nature of the test substance (i.e., due to its inability to crystallize). In summary, TNPP was cooled at 3 °C intervals to determine, in duplicate, the temperature that TNPP did not visibly move within a given time period. The pour point result has an inherent uncertainty of ± 3 °C.

Source: TRS Inc. Charlottesville
Reliability: (1) valid without restriction

(39)

2.2 Boiling Point

Value: = 180 degree C at 4 hPa

Method: other
Year: 1994
GLP: no

Reliability: (4) not assignable
test report not available

(23)

Value: > 303 degree C
Decomposition: no

Method: OECD Guide-line 103 "Boiling Point/boiling Range"
Year: 2001
GLP: yes

Test substance: Tris-nonylphenyl phosphite (TNPP; CAS#26523-78-4; Lot #173T110700 from Dover Chemical Corporation): Purity: 99.8%

Remark: The initial (T_{initial}) and final (T_{final}) hot stage temperatures were 303 °C and 304 °C respectively. TNPP was observed to boil only for the first 1 to 2 seconds of heating, and then boiling stopped. Conclusion: From these results it was concluded that the boiling point of TNPP was greater than 300 °C at 102 ± 1 kPa.

In the initial report, the result is expressed as >300°C because EPA HPV guidelines and Canadian CEPA guidelines indicate that boiling points above 300°C do not need to be specified.

The laboratory proposed to issue an addendum since the original wording indicates that TNPP boiled, but it was really a minor impurity in the test substance that boiled, not the test substance itself. The boiling point could be thus restated at >303°C (hot stage temperature).

Reliability: (1) valid without restriction

(35)

2.3 Density

Type: relative density
Value: = .98 g/cm³ at 20 degree C

Method: other
Year: 1994
GLP: no

Reliability: (2) valid with restrictions
test report not available

(10) (23)

2.4 Vapour Pressure

Value: = .00008 hPa at 20 degree C

Method: other (measured)
Year: 1991
GLP: no data

Reliability: (4) not assignable
test report not available

(4)

Value: = .00046 hPa at 20 degree C

Method: other (measured)
Year: 1997
GLP: no

Test substance: Tris-nonylphenyl phosphite (TNPP; CAS#26523-78-4; Source and purity not specified)

Source: TRS Inc. Charlottesville

Reliability: (2) valid with restrictions
Non GLP.

(32)

Value: .00047 hPa at 20 degree C

Method: (measured): ASTM D2879 (isoteniscope)
Year: 1997
GLP: no

Test substance: TNPP, no data on purity

Result: The value for 20 degrees C was extrapolated from the following measured values:

temp (°C)	Vp (Pa)
125	22.7
150	65.3
175	160
200	373
225	747
250	1533
275	2800
300	4666
325	8133
350	15330
375	65330

Initial decomposition occurred at 357 degrees C.

Reliability: (2) valid with restrictions
The isotenoscope method should not be used for substances with very low vapour pressure. The recommended range of this method is given between 10^2 and 10^5 Pa in OECD guideline 104. (32)

Value: 8 hPa at 245 degree C

Reliability: (4) not assignable (10)

Value: = .0000000005 hPa

Method: Synopsis of a report entitled "Fugacity Modeling to Estimate Transport Between Environmental Compartments for Tris-nonylphenyl phosphite (TNPP) (CAS Reg. No. 26523-78-4), dated November 10, 2001 for General Electric Company, Pittsfield, MA from Charles A. Staples, Ph.D., Assessment Technologies, Inc. Fairfax, VA.

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Estimation of Physical Properties

The SAR models for estimating physical properties and abiotic degradation were developed by the U.S. Environmental Protection Agency and Syracuse Research Corporation (Estimation Programs Interface for Windows, Version 3.05 or EPIWIN v.3.05) (Syracuse 2000). The models can be used to calculate key fugacity-based model input parameters including melting point, vapor pressure (submodel MPBPVP), octanol-water partition coefficient or Kow (submodel KOWWIN), and aqueous solubility (submodel WSKOWWIN). The calculation procedures are described in the program guidance and are adapted from standard procedures based on analysis of key structural features (Meylan and Howard, 1999a,b,c). Key assumptions and default parameters used in the models were developed under U.S. EPA guidance.

EPA uses the models for various regulatory activities.

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Atmospheric photo-oxidation potential was estimated using the submodel AOPWIN (Meylan and Howard, 2000). The estimation methods employed by AOPWIN are based on the SAR methods developed by Dr. Roger Atkinson and co-workers (Meylan and Howard, 2000a). The SAR methods rely on structural features of the subject chemical. The model calculates a second-order half-life with units of cm³/molecules-sec. Photo-degradation based on atmospheric photo-oxidation is in turn based on the second order rate of reaction (cm³/molecules-sec) with hydroxyl radicals (HO radical), assuming first-order kinetics and an HO radical concentration of 1.5 E+6 molecules/cm³ and 12 hours of daylight. Pseudo-first order half-lives (t_{1/2}) were then calculated as follows: t_{1/2} = 0.693 / [k_{phot} x HO radical x 12-hr / 24-hr].

Estimation of Environmental Distributions

The fugacity-based distribution model was based on the Trent University Modeling Center's EQUilibrium Concentration model (EQC) Level 3 model, version 1.01. These models are described in Mackay et al. (1996a,b). Fugacity-based modeling is based on the "escaping" tendencies of chemicals from one phase to another. For instance, a Henry's Law constant calculated from aqueous solubility and vapor pressure is used to describe the "escape" of a chemical from water to air or vice versa as it seeks to attain equilibrium between the phases. The key physical properties required as input parameters into the model are melting point, vapor pressure, octanol-water partition coefficient (K_{ow}), and aqueous solubility. The model also requires estimates of first-order half-lives in air, water, soil, and sediment. An additional key input parameter is loading or emissions of the chemical into the environment. The default assumption was used here, which assumes equal releases to air, water, and soil. The model was run using the chemical specific parameters to obtain estimates of the chemical distributions between environmental compartments.

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statement with a calculated log Kow value of 21.6 was provided (Reimer, 2001d). This expert statement was reviewed and is correct. Using the EPIWIN models described above, additional parameters were estimated. They include a water solubility of 1.3 E-15 mg/L, a vapor pressure of 5 E-12 Pa, an atmospheric half-life of 5.07 hours, water and soil biodegradation half-lives of 900 hours, and a sediment half-life of 3600 hours. Excepting the water and soil biodegradation half-lives, these values were all used in the distribution modeling. Hydrolysis is the dominant fate process in water and would be equally so in soil, so a 14 hour half-life was used in those compartments. The results of the distribution modeling (assuming equal emissions to air, water, and soil) were: Air 1.4 %, Water 4.5%, Soil 5.6%, and Sediment 88.6%.

Reliability: (3) invalid
Results were obtained that are out of the range of the definition domain of the QSAR model (44)

2.5 Partition Coefficient

Partition Coeff.: octanol-water
log Pow: 19.918
Method: other (calculated)
Remark: Very high LogP, unrealistic in nature
Reliability: (3) invalid (9)

Partition Coeff.: octanol-water
log Pow: = 21.6
Method: other (calculated)
Year: 2001
GLP: yes
Remark: It was not appropriate to conduct the Partition Coefficient test as TNPP was shown to be hydrolytically unstable. The solubility of TNPP in water was too low for it to be detected by the analytical method. Log Pow was 21.6 ± 0.6 .
Source: TRS Inc. Charlottesville
Reliability: (3) invalid
The reliability of calculation methods decreases as the complexity of the compound under study increases. Here, TNPP could be classified as a rather complex molecule with a high molecular weight and several functional groups. The domain of application of Kow calculation methods is characterised in terms of chemical structures. For example, some calculation programs cannot be applied to the estimation of Kow for phosphorus compounds including phosphites. Second, the domains of the models is also restricted by the log Kow range of their applicability. In general, clear estimates can be expected in the region of log Kow 0-5. Some programs have shown good estimates for compounds with log Kow > 5 but estimates for log Kow around 10 or above should be considered rather as qualitative than quantitative information (TGD, Part III, Chapter 4).

2.6.1 Solubility in different media

Solubility in: Water
Value: < .6 mg/l at 24 degree C
Descr.: of very low solubility

Method: OECD Guide-line 105
Year: 2001
GLP: yes
Test substance: Tris-nonylphenyl phosphite (TNPP; CAS#26523-78-4; Lot #173T110700 from Dover Chemical Corporation): Purity: 99.8%

Remark: The water solubility was determined by HPLC and the limit of detection was estimated at 0.6 mg/L. Temperature reported was 24 ± 1 °C.

Source: TRS Inc. Charlottesville
Reliability: (1) valid without restriction

(40)

Solubility in: Water
Value: = 0 mg/l

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Result: The following measured data were reported and used in the fugacity-based distribution modeling. TNPP undergoes hydrolysis with half-lives of 13 to 14 hours at pH values of 4 to 9 (at 22 °C) (Reimer, 2001a), has a melting point averaging 6 °C (Reimer, 2001b), and a water solubility of <0.6 mg/L (24 °C) (Reimer, 2001c). In addition, an expert statement with a calculated log Kow value of 21.6 was provided (Reimer, 2001d). This expert statement was reviewed and is correct. Using the EPIWIN models described above, additional parameters were estimated. They include a water solubility of 1.3 E-15 mg/L, a vapor pressure of 5 E-12 Pa, an atmospheric half-life of 5.07 hours, water and soil biodegradation half-lives of 900 hours, and a sediment half-life of 3600 hours. Excepting the water and soil biodegradation half-lives, these values were all used in the distribution modeling. Hydrolysis is the dominant fate process in water and would be equally so in soil, so a 14 hour half-life was used in those compartments. The results of the distribution modeling (assuming equal emissions to air, water, and soil) were: Air 1.4 %, Water 4.5%, Soil 5.6%, and Sediment 88.6%.

Source: TRS Inc. Charlottesville
Reliability: (4) not assignable

(44)

Value: < 100 mg/l at 20 degree C
pH value: = 5
Conc.: 200 g/l degree C

Method: other
Year: 1991
GLP: no data

Reliability: (4) not assignable
test report not available

(3)

2.7 Flash Point

Value: = 183 degree C
Type: closed cup

Method: other
Year: 1993
GLP: no

Reliability: (2) valid with restrictions
test report not available

(21)

Value: = 195 degree C
Type: closed cup

Year: 1991

GLP: no data
Reliability: (2) valid with restrictions
test report not available (4)

Value: 207 degree C
Type: closed cup

Method: Pensky - Martin
Year: 1978
GLP: no
Test substance: TNPP, no data on purity

Reliability: (2) valid with restrictions (10) (33)

2.8 Auto Flammability

Value: = 268 degree C

Method: other
Year: 1985
GLP: no data

Reliability: (4) not assignable
test report not available (31)

Value: 439 degree C

Reliability: (2) valid with restrictions (10)

Value: 440 degree C

Method: Setchkin method
Year: 1990
GLP: no
Test substance: TNPP, no data on purity

Reliability: (2) valid with restrictions (50)

2.11 Oxidizing Properties

Result: no oxidizing properties

Method: other
Year: 1993
GLP: no

Reliability: (4) not assignable
test report not available (21)

2.13 Viscosity

Value: 6000 mPa s (dynamic) at 25 degree C

Result: Viscosity vs. temperature:

Viscosity	Temperature
15000	15
6000	25
1300	40
525	50
395	55
250	60
115	70
80	80
50	90
32	100
21	110
18	120

Reliability: (2) valid with restrictions

(10)

3.1.1 Photodegradation

Type: water

Light source: Sun light

Method: (calculated): "Expert statement" based on EPA OPPTS 835.2210 (EPA, 1998)

Year: 2001

Test substance: Tris-nonylphenyl phosphite (TNPP; CAS#26523-78-4; Lot #173T110700 from Dover Chemical Corporation): Purity: 99.8%

Remark: The above-referenced EPA Guideline uses a two-tier approach to determine the photolytic stability of a test substance in water. The relevant requirements of this Guideline are as follows: In the Tier 1 experiment, a UV spectrum is recorded of the pure Test Substance dissolved in water. Based on the magnitude of absorbance over a specified wavelength range, it is determined if a Tier 2 experiment is required. In the Tier 2 experiment, the pure test substance is dissolved in water (at a concentration less than ½-saturation) and the solution is exposed to light for a specified length of time. The concentrations of the test substance over this period are measured by a suitable analytical method. These requirements could not be met for TNPP for the following reasons: 1. TNPP was found to be hydrolytically unstable: The TNPP half-life at pH 7 was approximately 13 h at 22 °C. 2. The solubility of TNPP in water was too low (less than 0.6 mg/L at 22 °C) for it to be detected by the analytical method, which utilized high-performance liquid chromatography with a UV detector monitoring 235 nm.

Therefore:

(1) It was not possible to conduct a Tier 1 experiment on TNPP because a pure aqueous TNPP solution could not be obtained due to its rapid hydrolysis, and, (2) It was not possible to conduct a Tier 2 experiment on TNPP because its water solubility was too low for it to be detected by the analytical method. In addition, the rate of hydrolysis

would most likely be significant relative to the rate of photolysis, and the hydrolysis reaction may interfere with the determination of the rate of any photolytic reaction.

Source: TRS Inc. Charlottesville
Reliability: (2) valid with restrictions

(37)

Type: air
DIRECT PHOTOLYSIS
Half-life t_{1/2}: = 5.1 hour(s)

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Source: TRS Inc. Charlottesville
Reliability: (2) valid with restrictions

(44)

3.1.2 Stability in Water

Type: abiotic
t1/2 pH4: = 13 - 14 hour(s) at 22 degree C
t1/2 pH7: = 13 - 14 hour(s) at 22 degree C
t1/2 pH9: = 13 - 14 hour(s) at 22 degree C

Method: OECD Guide-line 111 "Hydrolysis as a Function of pH"
Year: 2001
GLP: yes
Test substance: Tris-nonylphenyl phosphite (TNPP; CAS#26523-78-4; Lot #173T110700 from Dover Chemical Corporation): Purity: 99.8%

Remark: An experiment was conducted where TNPP was dissolved in buffers (pH 4, 7, and 9) with CH₃CN co-solvent (1/1; v/v). These solutions were placed in the injector tray of an HPLC-DAD (high-performance liquid chromatography with UV diode array detection) instrument, at 22 °C, and were repeatedly injected overnight. The observed (relative) Concentrations of TNPP decreased with time as hydrolysis occurred at pH 4, 7 and 9. From these results, the hydrolysis half-life of TNPP was estimated to be between 13 and 14 hours at 22 °C at pH 4, pH 7, and pH 9. There was no difference in the half-lives at pH 4, pH 7, and pH 9. Based on these results at 22 °C, it was concluded that the half-lives of TNPP under the conditions of the OECD Preliminary Test would be less than 13 hours (because Reaction rates generally increase with increasing temperature), and that TNPP would be classed as 'hydrolytically unstable' under these OECD test conditions (pH 4, 7, and 9; 50 °C). Further experimentation to investigate TNPP hydrolysis rates was not appropriate, due to 1) the maximum co-solvent concentration of 1 % allowed by OECD, and 2) the requirement for a much higher co-solvent concentration to detect TNPP in aqueous solution, due to its relatively low water solubility. In summary, TNPP was classified hydrolytically unstable under the OECD 111 Guidelines test conditions of pH 4, pH 7, and pH 9 at 50 °C for 5 days.

Source: TRS Inc. Charlottesville
Reliability: (1) valid without restriction

(38)

3.3.1 Transport between Environmental Compartments

Type: fugacity model level III
Media: air/water/soil/sediment

Method: Synopsis of a report entitled "Fugacity Modeling to Estimate Transport Between Environmental Compartments for Tris-nonylphenyl phosphite (TNPP) (CAS Reg. No. 26523-78-4), dated November 10, 2001 for General Electric Company, Pittsfield, MA from Charles A. Staples, Ph.D., Assessment Technologies, Inc. Fairfax, VA.

Below are the results of fugacity-based distribution modeling conducted for tris-nonylphenyl phosphite (TNPP). A number of study reports containing data needed for the modeling were provided and used. Below is a brief synopsis of physical-property and environmental fate modeling, fugacity-based distribution modeling, modeling results for TNPP, plus a few comments on the studies that were used.

Fugacity-based Distribution Modeling

Introduction

Fugacity-based distribution modeling requires several physical properties and fate characteristics as model inputs. Property estimation programs were used to obtain estimates of any physical property or fate characteristic (e.g., atmospheric photo-oxidation and biodegradation) for which data were not provided. To estimate the physical properties and fate characteristics, several models were employed. The models were based on structure-activity relationships (SAR) and were used to obtain aqueous solubility and vapor pressure for TNPP. SAR models were also used to estimate hydroxyl radical mediated atmospheric photo-oxidation for TNPP.

Estimation of Physical Properties

The SAR models for estimating physical properties and abiotic degradation were developed by the U.S. Environmental Protection Agency and Syracuse Research Corporation (Estimation Programs Interface for Windows, Version 3.05 or EPIWIN v.3.05) (Syracuse 2000). The models can be used to calculate key fugacity-based model input parameters including melting point, vapor pressure (submodel MPBPVP), octanol-water partition coefficient or Kow (submodel KOWWIN), and aqueous solubility (submodel WSKOWWIN). The calculation procedures are described in the program guidance and are adapted from standard procedures based on analysis of key structural features (Meylan and Howard, 1999a,b,c). Key assumptions and default parameters used in the models were developed under U.S. EPA guidance. EPA uses the models for various regulatory activities.

Estimation of Environmental Fate

Atmospheric photo-oxidation potential was estimated using the submodel AOPWIN (Meylan and Howard, 2000). The estimation methods employed by AOPWIN are based on the SAR methods developed by Dr. Roger Atkinson and co-workers (Meylan and Howard, 2000a). The SAR methods rely on structural features of the subject chemical. The model

calculates a second-order half-life with units of cm³/molecules-sec. Photo-degradation based on atmospheric photo-oxidation is in turn based on the second order rate of reaction (cm³/molecules-sec) with hydroxyl radicals (HO radical), assuming first-order kinetics and an HO radical concentration of 1.5 E+6 molecules/cm³ and 12 hours of daylight. Pseudo-first order half-lives (t_{1/2}) were then calculated as follows: $t_{1/2} = 0.693 / [k_{\text{phot}} \times \text{HO radical} \times 12\text{-hr} / 24\text{-hr}]$.

Estimation of Environmental Distributions

The fugacity-based distribution model was based on the Trent University Modeling Center's Equilibrium Concentration model (EQC) Level 3 model, version 1.01. These models are described in Mackay et al. (1996a,b). Fugacity-based modeling is based on the "escaping" tendencies of chemicals from one phase to another. For instance, a Henry's Law constant calculated from aqueous solubility and vapor pressure is used to describe the "escape" of a chemical from water to air or vice versa as it seeks to attain equilibrium between the phases. The key physical properties required as input parameters into the model are melting point, vapor pressure, octanol-water partition coefficient (K_{ow}), and aqueous solubility. The model also requires estimates of first-order half-lives in air, water, soil, and sediment. An additional key input parameter is loading or emissions of the chemical into the environment. The default assumption was used here, which assumes equal releases to air, water, and soil. The model was run using the chemical specific parameters to obtain estimates of the chemical distributions between environmental compartments.

Common Features of the Models

All of the models use the structure of the molecule to begin performing the calculation. The structure must be entered into the model in the form of a SMILES notation or string (Simplified Molecular Input Line Entry System). It is a chemical notation system used to represent a molecular structure by a linear string of symbols. The SMILES string allows the program to identify the presence or absence of various structural features that control aspects of the submodels. The models do contain structures and SMILES strings for about 100,000 compounds, accessible via Chemical Abstracts Service (CAS) Registry number.

Result:

The following measured data were reported and used in the fugacity-based distribution modeling. TNPP undergoes hydrolysis with half-lives of 13 to 14 hours at pH values of 4 to 9 (at 22 °C) (Reimer, 2001a), has a melting point averaging 6 °C (Reimer, 2001b), and a water solubility of <0.6 mg/L (24 °C) (Reimer, 2001c). In addition, an expert statement with a calculated log K_{ow} value of 21.6 was provided (Reimer, 2001d). This expert statement was reviewed and is correct. Using the EPIWIN models described above, additional parameters were estimated. They include a water solubility of 1.3 E-15 mg/L, a vapor pressure of 5 E-12 Pa, an atmospheric half-life of 5.07 hours, water and soil biodegradation half-lives of 900 hours, and a sediment half-life of 3600 hours. Excepting the water and soil biodegradation half-lives, these values were all used in

the distribution modeling. Hydrolysis is the dominant fate process in water and would be equally so in soil, so a 14 hour half-life was used in those compartments. The results of the distribution modeling (assuming equal emissions to air, water, and soil) were: Air 1.4 %, Water 4.5%, Soil 5.6%, and Sediment 88.6%.

Source: TRS Inc. Charlottesville
Reliability: (2) valid with restrictions

(44)

3.4 Mode of Degradation in Actual Use

Remark: The product is not stable in presence of water and moisture. It undergoes the hydrolysis. The kinetic depends on pH, in acid conditions the hydrolysis is faster than in basic condition.

Source: GREAT LAKES CHEMICAL ITALIA MILAN
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

Reliability: (4) not assignable

3.5 Biodegradation

Type: aerobic
Inoculum: activated sludge, non-adapted
Concentration: 15.4 mg/l related to DOC (Dissolved Organic Carbon)
Contact time: 28 day(s)
Degradation: < 4 %
Result: under test conditions no biodegradation observed
Kinetic: 28 day(s) < 4 %
Control Subst.: Sodium acetate
Kinetic: 14 day(s) = 68 %
28 day(s) = 72 %
Deg. product: not measured

Method: OECD Guide-line 301 D "Ready Biodegradability: Closed Bottle Test"

Year: 2001
GLP: yes

Test substance: Tris-nonylphenyl phosphite (TNPP; CAS#26523-78-4; Lot #173T110700 from Dover Chemical Corporation): Purity: 99.8%

Remark: Note that TNPP has a calculated octanol water partition coefficient (log10 basis) of 21. This very high log Kow value implies a water solubility well below the estimated aqueous solubility of 0.6 mg/L TNPP. In fact, the aqueous solubility of TNPP may be below 1 microgram per liter, in the nanogram per liter range. Upon entry into water, only very small amounts of TNPP will solubilize in water. Therefore TNPP is essentially unavailable for biodegradation. Any solubilized TNPP will hydrolyze to phosphorous acid and nonylphenol. Nonylphenol has been shown to biodegrade under aerobic conditions.

TNPP was not readily biodegradable. The test substance did not meet the criterion of greater than a 60 % of the ThODNO3 within a 14-d window over the 28-d test period. The amount of biodegradation of the test substance at 28 days was less than 4 %. The summary of test conditions, summary

of dissolved oxygen determinations, and percent degradation calculations are as follows:

Table 3.5.1. Summary of Test Conditions

Parameter	Test Condition
Test type	Static
Duration	28 days
Inoculum	Polyseed
Temperature	20 ± 1 °C
DO Determination Method	Electrode
Initial Dissolved Oxygen	7.8 mg/L
Test vessel	BOD bottles
Test volume	300 mL
Replicates	Two at each of five time intervals
Aeration	None
Controls	<ol style="list-style-type: none"> 1. Test control (inoculum blank) 2. Procedure control (Sodium acetate plus inoculum) 3. Toxicity control (Sodium acetate, TNPP and inoculum)
Nominal TNPP concentration	15.4 ± 1.0 mg/L
Criterion for Ready Biodegradability	60% ThOD in 14-d window within 28 days

Table 3.5.2. Summary of Dissolved Oxygen Determination

Bottle Contents	Calculation Data	Dissolved Oxygen mg O ₂ /L after n days				
		0	7	14	21	28
Test control (Inoculum blank)	C ₁	7.8	7.8	7.2	7.2	6.9
	C ₂	7.8	7.9	7.1	7.2	6.9
	$m_b = (C_1 + C_2) / 2$	7.8	7.8	7.1	7.2	6.9
Test Substance (TNPP)	a ₁	7.8	7.8	7.2	6.5	5.8
	a ₂	7.9	7.8	7.2	7.3	4.9
Reference Substance (Sodium Acetate)	r ₁	7.9	3.9	3.2	3.5	3.1
	r ₂	7.8	4.0	3.7	3.2	2.8
Toxicity control:	tox ₁	7.8	3.5	4.1	3.5	3.0

Table 3.5.3. Percent Degradation Calculations

Calculations (as per OECD 301D Method)	Test Day			
	7	14	21	28
$(m_{b(0)} - m_{b(28)}) < 1.5 \text{ mg/L}$				0.9
Inoculum Control Validity Criterion				PASS
Test Substance (TNPP)				
$(m_b - a_1)$	0.0	-0.1	0.7	1.1
$(m_b - a_2)$	0.0	-0.1	-0.1	2.0
$\% Da_1 = 100 \times (m_b - a_1) / (\text{TNPP mg/L} \times \text{ThOD})$	0	0	2	2
$\% Da_2 = 100 \times (m_b - a_2) / (\text{TNPP mg/L} \times \text{ThOD})$	0	0	0	5
$\% D_{\text{mean}} = (Da_1 + Da_2) / 2$	0	0	1	4
Ready Biodegradable ($\%D_{\text{mean}} > 60\%$) (YES or NO)				NO
Check Final pH (> 6.0)				7.6
Reference Substance (Sodium Acetate)				
$(m_b - r_1)$	4.0	4.0	3.7	3.8
$(m_b - r_2)$	3.8	3.4	4.0	4.1
$\% Dr_1 = 100 \times (m_b - r_1) / (\text{Sodium acetate mg/L} \times \text{ThOD})$	73	72	67	70
$\% Dr_2 = 100 \times (m_b - r_2) / (\text{Sodium acetate mg/L} \times \text{ThOD})$	70	63	73	74
$\% Dr_{\text{mean}} = (Dr_1 + Dr_2) / 2$	72	68	70	72
$\% Dr_{\text{mean}}$ must be $\geq 60\%$ within 14 days		PASS		
Toxicity Check				
$(m_b - \text{tox}_1)$	4.4	3.1	3.8	3.9
$\% Dr_1 = 100 \times (m_b - r_1) / (\text{Sodium acetate mg/L} \times \text{ThOD})$	80	56	69	71
$\% D_{\text{inhibition}} = 100 \times (\%Dr_{\text{mean}} - \% D_{\text{tox}_1}) / \% Dr_{\text{mean}}$	-12	17	2	2
Toxic if $\% D_{\text{inhibition}} > 25\%$ within 14 days		PASS		

Note: Concentration was 15.4±1.0 mg/L.

Source: TRS Inc. Charlottesville
Reliability: (2) valid with restrictions

(17)

Type: aerobic
Result: Water/Soil & Sediment t1/2 = 900 & 3600 hours (calculated)

Method: Synopsis of a report entitled "Fugacity Modeling to Estimate Transport Between Environmental Compartments for Tris-nonylphenyl phosphite (TNPP) (CAS Reg. No. 26523-78-4), dated November 10, 2001 for General Electric Company, Pittsfield, MA from Charles A. Staples, Ph.D., Assessment

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Source:

TRS Inc. Charlottesville

Reliability:

(2) valid with restrictions

(44)

Type:

aerobic

Inoculum:

activated sludge

Concentration: 18.1 mg/l related to Test substance
Degradation: 1 % after 29 day(s)
Result: under test conditions no biodegradation observed
Control Subst.: Benzoic acid, sodium salt
Kinetic: 7 day(s) 71 %
 20 day(s) 86 %
Deg. product: not measured

Method: OECD Guide-line 301 B "Ready Biodegradability: Modified Sturm Test (CO2 evolution)"
Year: 1994
GLP: no

Remark: Deviation from the guideline: only one CO2 scrubber was used. Theoretically, a solution of 0.05 M NaOH is sufficient to trap at least twice more CO2 than the maximum ThCO2 which can evolve in each test bottle (including CO2 possibly evolved from the bacteria, e.g. endogenous respiration). Moreover, experimentally it was confirmed that no measurable CO2 carry over has ever occurred with the scrubbers used.

Result: The test substance is not biodegradable in this test.

The controls of reference and reference + test substance together meet the specifications for ready biodegradability. Therefore, it can be concluded that the test substance has no inhibitory effect on the bacteria.

Biodegradation results (%)

Day	Reference	Ref.+Test Subst.	TS1	TS2
0	0	0	0	0
3	58	48	-4	-4
7	71	62	-3	-3
10	78	68	-3	-2
13	80	74	-2	-1
17	90	83	0	2
20	86	85	0	0
24			0	2
28			-3	2
29			1	1

Test condition: Reference substance: sodium benzoate.

Activated sludge collected from the sewage treatment plant of CH-4153 Reinach on 14/02/94. The pH after collection was 7.0.

The preparation was carried out according to the method described in the guideline.

Temperature = 22+/-2°C

Air: carbon dioxide free air at 25 mL/min

Flasks 1&2: test substance and inoculum
 Flasks 3&4: test medium and inoculum (inoculum blank)
 Flask 5: reference substance and inoculum (procedure control)
 Flask 6: reference substance, test substance and inoculum (control of toxicity and inhibition of the bacteria's activity by the test substance).

Test substance (in duplicates): 18.1 mg/L=15.3 mg ThOC/L except in flask 6 where test substance concentration = 15.3 mg ThOC/L.

Reference substance: 15 mg DOC/L

Before application, the inoculum was pre acclimated to the test medium overnight.

Preparation of the test substance: a stock solution of 1.36 g test substance in 10 mL dichloromethane was prepared. From this stock solution, for each replicate, 27.2 mg (200 µL) were applied onto a filter paper as small drops. After the filter paper was completely dry (no remaining of dichloromethane was present), it was cut to small pieces (10-15) and added to the test medium. Thereafter, the medium volume was completed to 1.5L with 300 mL water and the flasks were immediately connected to the CO2 scrubber. Within a few hours the filter paper was homogeneously distributed in the test medium (so that it could not be seen anymore).

The evolved CO2 was measured at 0, 3, 7, 10, 13, 17, 20, 24, 28 and 29 days.

Reliability: (2) valid with restrictions

(8)

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: static
Species: Rainbow trout (*Oncorhynchus mykiss*) (Spring Valley Trout Hatchery, Langley, British Columbia)
Exposure period: 96 hour(s)
Unit: mg/l
Analytical monitoring: yes
NOEC: > 100 - calculated
LC50: > 100 - measured/nominal

Method: OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year: 2001
GLP: yes
Test substance: Hydrolyzed solution of tris-nonylphenyl phosphite (TNPP; CAS # 26523-78-4; from Dover Chemical Corporation): Purity, 99.8%)

Remark: Note: TNPP is known to rapidly hydrolyze in water to NP and phosphorus acid. Therefore, relevant OECD guidelines regarding hydrolytically unstable compounds were employed to conduct aquatic toxicity tests with TNPP. An initial target concentration of 100 mg TNPP/L was used to prepare stock solutions of TNPP's hydrolysis breakdown products. One hundred units of TNPP will yield about 96 units of NP through hydrolysis. Thus, theoretically, 96 mg NP/L could be formed via hydrolysis in the solutions prepared from 100 mg TNPP/L. The theoretical concentration of 96 mg NP/L is well above the measured aqueous solubility of 6 mg/L and above concentrations of NP known to be acutely toxic. However, as noted below, no toxicity was observed in this

study that used the stock solution of TNPP breakdown products.

The absence of toxicity to aquatic organisms from solutions of hydrolysis breakdown products is expected based on the known and calculated physical properties of TNPP. TNPP has a calculated octanol water partition coefficient (log₁₀ basis) of 21. This very high log K_{ow} value implies a water solubility well below the estimated aqueous solubility of 0.6 mg/L TNPP. In fact, the aqueous solubility of TNPP may be below 1 microgram per liter, in the nanogram per liter range. Upon entry into water, only very small amounts of TNPP will solubilize in water. It is only dissolved TNPP that will hydrolyze to NP and phosphorus acid. Therefore, if less than 1 microgram of TNPP gets solubilized into one liter of water, less than 1 microgram per liter of NP will be formed. This concentration of NP is well below all available acute toxicity data. Therefore, the absence of toxicity to aquatic organisms was expected using the solutions prepared according to relevant OECD guidelines. Thus, this study should be considered valid without restrictions.

Test conditions: The fish were held 33 days before initiating the test on TNPP. Mortality in the stock culture was less than 0.1 % the week prior to test initiation. The fish were fed a daily ration of trout chow equal to 5 % of their body weight. The fish were not fed 24 h prior to test initiation or during the test. The dilution water was dechlorinated City of Calgary tap water (charcoal filtered and aerated). The dilution water had a hardness of 198 mg CaCO₃/L, alkalinity of 140 mg CaCO₃/L, pH of 7.6, and a conductance of 446 ms/cm. The test solutions were prepared from a stock solution initially containing 100.0 mg/L of TNPP. TNPP is not water-soluble (< 0.6 µg/mL at 24 ± 1 °C). The major hydrolysis product, nonylphenol, is also sparingly soluble in water. However, phosphorous acid released upon hydrolysis of TNPP is water-soluble. An initial concentration of 100 mg/L was selected for the test. This was considered reasonable in light of the low water solubility of TNPP and nonylphenol. The substance (2 g) was weighed onto a glass Petri dish. The dish was then placed into a 22-L plastic pail fitted with a polyethylene liner and containing 20 L of dilution water (100.0 mg/L nominal concentration). Two separate stock solutions were prepared (40-L volume required for the test). The solutions were gently aerated for 78 h at room temperature (20 ± 2 °C). The supernatants containing the hydrolysis products of TNPP were then decanted for preparation of the test solutions. The stock solutions and 200 L of dilution water were cooled to the test temperature overnight in a controlled environment chamber (15 °C with aeration). At test initiation, dissolved oxygen, temperature, and pH ranged from 8.7 to 9.2 mg/L (98 % to 100 % saturation), 14 to 16 °C, and 7.7 to 8.0 units, respectively. At test termination, the temperature and pH of the test solutions were 15 °C and 7.8, respectively. Dissolved oxygen levels ranged from 6.2 to 6.8 mg/L (69 to 75 % saturation). Samples from each test vessel and the control were collected at test initiation and termination in 50-mL polypropylene centrifuge tubes. The samples were frozen and

stored in darkness at -19 ± 2 °C and then shipped frozen on ice to Reimer Analytical & Associates Inc. (Vancouver, BC, Canada) for analysis. The test substance was unstable in water and hydrolyzed to form three molecules of nonylphenol and one molecule of phosphorous acid. A 100 mg/L solution of TNPP will yield upon complete hydrolysis 12 mg/L phosphorous and 96 mg/L nonylphenol. The additional mass is a result of hydrolysis (addition of hydrogen and oxygen from water). The samples were not analyzed for the parent compound, TNPP, because it is not soluble in water. The samples were also not analyzed for phosphorous acid because it is unstable in water and not toxic to rainbow trout at low concentrations (< 1.2 mg/L; nominal concentration of phosphorous acid following complete hydrolysis) in laboratory dilution water at the test pH of 8.2 to 8.3. Phosphorous acid will degrade to phosphoric acid and phosphate over time. The test solutions were analyzed for nonylphenol.

Summary of test conditions:

Table 4.1.1. Summary of Test Conditions

Parameter	Test Condition
Test type	Static
Duration	96 h
Test organism / size (weight / age)	Rainbow trout (<i>Oncorhynchus mykiss</i>) / 4.0±1.0 cm (0.3 to 0.5 g / approx. 50 days post hatch)
Photoperiod	8-h dark and 16-h light
Light intensity	136 lux at the water surface
Temperature	15 \pm 2 °C
Dissolved oxygen	98 % to 102 % saturation
Feeding	None
Test vessel	22-L pails fitted with polyethylene liners
Test volume	20 L
Loading density	10 fish per test vessel (< 1.0 g fish per liter)
Replicates	One
Aeration	Not aerated
Controls	Dilution water
Nominal concentrations	0, 1.6, 3.1, 6.3, 12.5, 25.0, 50.0, and 100.0 mg/L
Measured concentrations	Not measured (TNPP is insoluble in water) Hydrolysis product (nonylphenol) measured
Criterion for effect	Death
Calculated toxicity values	LC50 at 24, 48, 72, and 96 h

Results: The TNPP hydrolysis products were not lethal to trout under the conditions in which the stock solution was

prepared, diluted and tested. There were no signs of stress or unusual behavior exhibited by the fish in any of the treatment concentrations. No fish died at any concentration at any time point. The highest non-lethal concentration tested was set as greater than or equal to the 100.0 mg/L of TNPP hydrolysis products. LC50 was > 100 at 24, 48, 72 and 96h.

Source: TRS Inc. Charlottesville
Reliability: (2) valid with restrictions

(16)

Type: static
Species: Brachydanio rerio (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l
Analytical monitoring: no
NOEC: < 10 -
LC50: < 10 -
LC100: = 10 -
LC50 (48h): = 16 -
LC50 (24h): = 29 -

Method: Directive 84/449/EEC, C.1 "Acute toxicity for fish"
Year: 1992
GLP: no
Test substance: Irgafos TNPP, purity > 94%

Result: The oxygen content ranged from 89-97% at 24 hours, 68-83% at 48 hours, and 60-76% at 72 hours. In the pretest, 10 mg/L had no effect to the fish after 96 hours of exposure. In the main test, 10 mg/L showed no effect to the fish after 48 hours. However, the oxygen concentration in the water was determined to be low at 48 hours and a gentle aeration was started at this time. After 72 hours of exposure with the test substance, all fish were dead.

A small part of the test substance was swimming at the surface of the test vessels at all test times and concentrations.

Values calculated: LC50(96h)<10 mg/L ; LC50(72h)<10 mg/L ; LC50(48h)=16 mg/L (95% CL 12-19 mg/L) ; LC50(24h)=29 mg/L (95% CL 23-36 mg/L).

Values observed: NOEC(48h)<10 mg/L ; LC0(72h)<10 mg/L ; LC100(72h)=10 mg/L.

Controls: mortalities in blank (0%) ; mortalities in vehicle (0%).

Test condition: Tested concentrations: 0, 10, 18, 32, 58, 100 mg/l
Blank = dechlorinated tap water
Vehicle = 4 mg Alkylphenol-Polyglycol-Ether (ARKOPAL)

pH: 7.3 - 7.9

temp: 22+/-1°C

Dechlorinated tap water ; hardness: 171 mg CaCO3/l

Gentle aeration after 48 hours exposure. Fluorescent light, 16 hours daily.

Stock solution contained 4 g. test substance and 160 mg Alkylphenol-Polyglycol-Ether (ARKOPAL) were mixed with and made up to 2000 mL with water.

10 fish were disposed per aquarium. Fish were acclimated 125 days prior the test. They were adapted to test medium 24 hour prior testing and no food was delivered 24 hours prior to exposure.

Reliability:

(2) valid with restrictions

The tested concentrations were probably very far above the actual water solubility of the substance. No analytical follow-up of the test concentrations was performed. As there was no equilibration time to allow dissolution of the substance during the preparation of the test concentration, it is not even clear that the maximum solubility in the test medium was achieved. The report mentions that undissolved substance was observed at all test concentrations.

All fish died at the lowest test concentration during aeration of the test system at t = 48 h. No LC50 could be estimated.

(2)

Type: static
Species: Leuciscus idus (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l
Analytical monitoring: no
LC0: < 5.8 -
LC50: 7.1 - calculated
LC100: 10 -
Method: DIN-Vorschrift 38412-L15
Year: 1988
GLP: no
Test substance: other

Result: The LC-50 values were calculated according to Berkson, JASA 48 (1953), 569-599

Values are based on nominal concentrations.

[mg/L]	Mortalities (number of dead fish)	
	24h	48h
Blank	0	0
Vehicle	0	0
5.8	1	1
10	10	10
18	10	10
32	10	10
58	10	10
100	10	10

Different symptoms were observed at the different test concentrations: moderate effects on swimming behaviour and on fish equilibrium were observed after 24 and 48 hours at

the concentration of 5.8 mg/L. Slight effects on the respiratory function has been observed after 48 hours, at 5.8 mg/L.

Test condition: Temperature: 20+/-1°C

Highest vehicle concentration 950 mg/L

Fish size: 35-50 mm (average=44mm) ; weight=0.59g (0.29-0.85g). Loading=0.39 g/L.

Acclimation phase lasted 22 days.

Test volume=15L

Fish were not fed for 3 days prior to exposure

Stock solution: 5 g TK 10417 were dissolved in made up to 50 mL with DMF.

10 fish were disposed per concentration and control, in each aquarium (20L filled with 15L dechlorinated tap water).

Hardness 254 mg CaCO₃/L (Ca/Mg = 4/1)

Gentle aeration during the test. Fluorescent light, 16 hours daily.

Test concentrations: 5.8 ; 10 ; 18 ; 32 ; 58 ; 100 mg/L.

950 mg DMF per liter water in the concentration used for the highest test concentration.

O₂, pH and temperature were measured at 0, 24 and 48 hours. O₂>91% saturation ; pH=7.9-8.2

Test substance: TK 10417 (TNPP)

Reliability: (2) valid with restrictions

(5)

4.2 Acute Toxicity to Aquatic Invertebrates

Type: static

Species: Daphnia magna (Crustacea)

Exposure period: 48 hour(s)

Unit: mg/l

Analytical monitoring: yes

NOEC: = .16 - measured/nominal

EC50: = .3 - measured/nominal

Method: OECD Guide-line 202

Year: 2001

GLP: yes

Test substance: Hydrolyzed solution of tris-nonylphenyl phosphite (TNPP; CAS # 26523-78-4; from Dover Chemical Corporation): Purity, 99.8%)

Remark: Note: TNPP is known to rapidly hydrolyze in water to NP and phosphorus acid. Therefore, relevant OECD guidelines were employed to conduct aquatic toxicity tests with TNPP.

An initial target concentration of 100 mg TNPP/L was used to prepare stock solutions of TNPP's hydrolysis breakdown products. One hundred units of TNPP will yield about 96 units of NP through hydrolysis. Thus, theoretically, 96 mg NP/L could be formed via hydrolysis in the solutions prepared from 100 mg TNPP/L. The theoretical concentration of 96 mg NP/L is well above the measured aqueous solubility of 6 mg/L and above concentrations of NP known to be acutely toxic. However, no NP was detectable in any test vessel or concentration, except for one vessel at the highest concentration and test initiation.

Toxicity to aquatic organisms from solutions of hydrolysis breakdown products is not expected based on the known and calculated physical properties of TNPP. TNPP has a calculated octanol water partition coefficient (log₁₀ basis) of 21. This very high log K_{ow} value implies a water solubility well below the estimated aqueous solubility of 0.6 mg/L TNPP. In fact, the aqueous solubility of TNPP may be below 1 microgram per liter, in the nanogram per liter range. Upon entry into water, only very small amounts of TNPP will solubilize in water. It is only dissolved TNPP that will hydrolyze to NP and phosphorus acid. In addition, TNPP is a viscous greasy or oily textured material. Daphnids are susceptible to physical effects, rather than general toxic effects to compounds like TNPP that have a very low aqueous solubility and density less than that of water. The observed toxicity to daphnia based on nominal concentrations is consistent with other measured values of toxicity of nonylphenol to this species. However, it is just as likely due to physical effects caused by the viscous and insoluble TNPP.

Test conditions: The test was initiated with young daphnids less than 24 h old from in-house cultures. The culture vessels were incubated in a temperature-controlled room at 20 + 2 °C, under an 8-h dark and 16-h light photoperiod. The light intensity at the water surface was 392 lux (provided by cool white florescent lights). Stock cultures were fed a 50:50 (v:v) mixture of a green alga (*Raphidocelis subcapitata*, formerly *Selenastrum capricornutum*) and a solution of yeast, alfalfa powder, and fermented trout chow three times weekly (Monday, Wednesday, and Friday). Culture vessels were cleaned on Monday and Friday and the culture turned over on Wednesday. On these days the number of adults and young were counted and recorded for each jar. Young for testing were collected from 3- to 4-week-old adults. Mortality in the stock culture was less than 1 % in the week prior to test initiation. Dilution water was dechlorinated City of Calgary tap water (charcoal filtered and aerated). The dilution water had a hardness of 188 mg CaCO₃/L, alkalinity of 100 mg CaCO₃/L, pH of 8.1, and conductivity of 421 ms/cm. These parameters were measured on May 9, 2001. The ratios of calcium-to-magnesium and sodium-to-potassium on a weight-to-weight basis were 3.4 and 4.0 respectively (sample collected for analyses on January 11, 2001). The concentration of dissolved oxygen was 8.2 mg/L (100 % saturation at the test temperature 20 + 1 °C corrected for altitude). The test solutions were prepared from a stock solution initially containing 100 mg of TNPP in 1 L of dilution water. TNPP is not water-soluble (< 0.6 µg/mL at

24 ± 1 °C). The major hydrolysis product, nonylphenol, is also sparingly soluble in water. However, phosphorous acid released upon hydrolysis of TNPP is water-soluble. The mass of TNPP selected for the test was based on initial attempts to get enough of the hydrolysis products in solution to be acutely toxic to *Daphnia magna*. The method detailed below provided a stock solution that was acutely lethal to *Daphnia magna*. TNPP (100 mg) was weighed onto a glass Petri dish. The dish and test substance were placed into a two-liter, glass Erlenmeyer flask containing 1 L of dilution water. A magnetic stir bar was added and the mouth of the flask sealed with Parafilm®. The test substance was gently stirred for 78 h at room temperature (20 ± 2 °C). The supernatant containing the hydrolysis products of TNPP was then decanted for preparation of the test solutions. A stock was prepared from the hydrolyzed TNPP solution by diluting 100 mL of the supernatant with 900 mL of dilution water (10.00 mg/L nominal). This solution was then serially diluted with laboratory dilution water to obtain the other eight test concentrations (5.00, 2.50, 1.25, 0.63, 0.31, 0.16, 0.08, and 0.04 mg/L). The dilution was done with 500-mL volumes of the test solutions and dilution water (dilution factor of 2). The concentrations were nominal values based on the total mass of TNPP initially added to the flask and hydrolyzed for 78 h (100.0 mg/L). The control was laboratory dilution water. Four 50-mL volumes of each test solution were dispensed to replicate test vessels (100-mL glass beakers) and an additional 50 mL was archived for chemical analysis. The remaining volume was for measurements of pH, dissolved oxygen, and temperature. The pH was measured with an Oakton® pH meter equipped with a combination glass electrode with temperature compensation. Dissolved oxygen was measured with a YSI 9501® probe equipped with temperature compensation connected to a Model 95 Dissolved Oxygen® meter. Temperature was measured with the probe connected to the dissolved oxygen meter. All instruments were calibrated daily against appropriate standards (Supporting Work Instructions 4.3.2.1 and 4.3.2.2, Quality Manual, HydroQual Laboratories Ltd.). The test organisms were *Daphnia magna* neonates (less than 24-h old). The daphnids were collected from the culture vessels and distributed to 30-mL plastic cups. The organisms were then added to the test vessels in a random fashion (final loading density of one organism per 10 mL of test solution). There were four replicates for each test concentration containing 5 daphnids. The replicates were labeled a, b, c, and d. The daphnids were not fed during the test. Beakers were placed on a tray and covered with a glass sheet. The test was conducted at conditions similar to the culture conditions. Beakers were placed in a temperature-controlled room to maintain the test temperature at 20 ± 2 °C. The light intensity at the water surface was 390 lux with an 8-h dark and 16-h light photoperiod. The test vessels were examined at 24 and 48 h, and the number of immobilized organisms recorded along with any observations of unusual behavior. Immobilisation was defined as the inability of a daphnid to swim within 15 sec after gentle prodding. At test termination, samples were collected for chemical analysis of the test substance and measurements of pH, dissolved oxygen, and temperature. Chemical analysis: Samples of the test and control

solutions were collected for chemical analysis in 50-mL polypropylene centrifuge tubes at test initiation and termination. The samples were frozen and stored in darkness at -19 ± 2 °C and then shipped, frozen on ice to Reimer Analytical & Associates Inc. (Vancouver, B.C., Canada) for analysis. The test substance was unstable in water and hydrolyzed to form three molecules of nonylphenol and one molecule of phosphorous acid. A 100 mg/L solution of TNPP will yield upon complete hydrolysis 12 mg/L phosphorous and 96 mg/L nonylphenol. The additional mass is a result of hydrolysis (addition of hydrogen and oxygen from water). The samples were not analyzed for the parent compound, TNPP, because it is not soluble in water. The samples were also not analyzed for phosphorous acid because it is unstable in water and not toxic to *Daphnia magna* at low concentrations (< 1.2 mg/L; nominal concentration of phosphorous acid following complete hydrolysis) in laboratory dilution water at the test pH of 8.2 to 8.3. Phosphorous acid will degrade to phosphoric acid and phosphate over time (The Merck Index, 1989). The samples of the test solutions were analyzed for the major hydrolysis product of TNPP, nonylphenol. Nonylphenol was only detected in the highest treatment at test initiation (0.3 mg/L based on the results of duplicate analyses; detection limit of 0.2 mg/L). Toxicity values were derived based on this measured concentration of nonylphenol. The test concentrations for toxicity values were derived from the single value for nonylphenol (starting value that was serially diluted by a factor of 2 to obtain the numerical values for the test concentrations, all of which were below the detection limit of 0.2 mg/L for nonylphenol).

Results: At test initiation the concentration of dissolved oxygen, temperature, and pH ranged from 8.2 to 8.3 mg/L (99 % saturation), 19 °C, and 8.1 to 8.3 units, respectively. At test termination, the concentration of dissolved oxygen, temperature, and pH ranged from 7.6 to 7.8 mg/L (96 to 100 % saturation), 21 °C, and 8.2 to 8.3 units, respectively. Dead organisms were considered immobilized. The organisms were not prodded at 24 h and observations were made based on the inability of the daphnid to swim within a 15-sec period after shaking the test vessel. The organisms were also not removed from the test vessel to verify death (visually checking for the presence or absence of a heartbeat). The test organisms were prodded at 48 h and removed from the test vessels for microscopic examination of the heart.

Results were as follows:

Table 4.2.1. Immobile and Dead Daphnids at 0, 24, and 48 h

Conc. (mg/L)	0 h				24 h				48 h				Averages (%)		
	a	b	c	d	a	b	c	d	a	b	c	d	0 h	24 h	48 h
control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.08	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.31	0	0	0	0	0	0	5	0	3	5	5	2	0	25	75
0.63	0	0	0	0	0	0	0	0	5	5	5	3	0	0	90

1.25	0	0	0	0	0	0	0	0	0	5	5	5	5	0	0	100
2.50	0	0	0	0	4	3	2	3	5	5	5	5	5	0	60	100
5.00	0	0	0	0	5	0	100	100								
10.00	0	0	0	0	5	0	100	100								

Note: Conc., nominal concentration of hydrolysis products; bold indicates the daphnids were confirmed dead.

Immobility after 24 h ranged from 60 to 100 % in the three highest test concentrations. The daphnids in the two highest treatments were confirmed dead (5.0 and 10.0 mg/L nominal concentrations). The 2.5 mg/L treatment was not lethal to *Daphnia* after a 24-h exposure. There were 5 immobile daphnids in one replicate of the 0.31 mg/L test concentration. The degree of immobility also increased with an increase in the test substance concentration. A greater degree of immobility was observed at 48 h. All daphnids were either immobile or dead in the top four test concentrations (1.25, 2.50, 5.00, and 10.00 mg/L). Ninety percent of the daphnids were immobilized in the 0.63 mg/L test concentration and seventy percent were immobilized in the 0.31 mg/L treatment. The daphnids were all alive and active in the lower test concentrations (0.02 to 0.16 mg/L). Toxicity values were derived based on nominal concentrations for the mixture of TNPP hydrolysis products. These nominal values were likely higher than actual concentrations because of the sparingly soluble nature of the test substance and hydrolysis products. The concentrations and 95 % confidence limits of the hydrolysis products that immobilized 50 % of the daphnids at 24 and 48 h were 2.2 mg/L (1.7 to 3.0 mg/L) and 0.3 mg/L (0.2 to 0.4 mg/L), respectively. The highest concentrations of hydrolysis products that produced no significant immobility relative to controls at 24 and 48 h were 1.25 and 0.16 mg/L, respectively (NOEC). The lowest concentrations of hydrolysis products that produced significant immobility relative to controls at 24 and 48 h were 2.50 and 0.31 mg/L, respectively (LOEC). The lowest concentrations that immobilized 100 % of the daphnids at 24 and 48 h were 5.00 and 1.25 mg/L, respectively. The highest concentrations that caused no immobility at 24 and 48 h were 1.25 and 0.16 mg/L. The degree of immobilization increased with an increasing concentration of the hydrolyzed test substance as expected (normal dose and response relationship). The toxic response and presence of detectable levels of the hydrolysis product in solution confirmed that the TNPP had undergone hydrolysis during preparation of the stock solution. TNPP is not soluble in water and the only major hydrolysis product is nonylphenol. Hence, nonylphenol is likely the toxic agent present in the test solutions.

Source: TRS Inc. Charlottesville

Reliability: (2) valid with restrictions

Due to analytical difficulties, this study should be considered valid, but used with care.

(15)

Type: static
Species: *Daphnia magna* (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l
Analytical monitoring: no

NOEC: = .058 -
EC50: = .42 -
EC100: > 1 -

Method: Directive 84/449/EEC, C.2 "Acute toxicity for Daphnia"
Year: 1992
GLP: yes
Test substance: TNPP, purity > 94% (Irgafos)

Result: Parts of the test substance were swimming on the surface of the water at 0.1 to 1.0 mg/l

Test condition: Test concentrations: 0.058, 0.1, 0.18, 0.32, 0.58, 1.0 mg/l
Hardness: 240 mg CaCO₃/l
temp: 22 +/- 2 °C
pH 7.8 - 8.0
DO: 98-100% saturation
Stock solution contained 2 mg/l TNPP
Solvent: alkylphenolpolyglycolether

Calculated amounts of the test material to produce the desired concentrations were added to the water and were homogeneously distributed. Values are based on the nominal concentrations. Parts of the test substance were visible on the surface of the water at concentrations of 0.1-1.0 mg/L. One day before the start of exposure, reproductive daphnia are separated from the young (0-24 hours old) by sieving all individuals through an 800 µm sieve. This procedure is repeated immediately prior to exposure and the young are retained for the test. The daphnia (4 replicates of 5 daphnia each) were then transferred into the beakers. Cultures of daphnia were maintained in glass vessels containing approximately 2.5 liters of reconstituted water and maintained at 20 +/- 1°C. The oxygen content ranged from 97 to 103%, the pH ranged from 7.8 to 8.0, and the water temperature was maintained at 21-24°C throughout the experiment. The EC-50 values were calculated according to the maximum likelihood method, probit model. EC-values were graphically determined on gaussian-logarithmic probability paper. The EC-50 values at 24 and 48 h were 2.6 and 0.42 mg/l, respectively. The LOEC at 48 h was 0.1 mg/l.

Reliability: (2) valid with restrictions

The tested concentrations were probably far above the actual water solubility of the substance. No analytical follow-up of the test concentrations was performed. As there was no equilibration time to allow dissolution of the substance during the preparation of the test concentration, it is not even clear that the maximum solubility in the test medium was achieved.

(1)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: Green algae (Raphidocelis subcapitata, formerly Selenastrum capricornutum)
Endpoint: growth rate
Exposure period: 72 hour(s)
Unit: mg/l
Analytical monitoring: yes
NOEC: = 100 - measured/nominal
LOEC: > 100 - measured/nominal

Method: OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year: 2001
GLP: yes
Test substance: Hydrolyzed solution of tris-nonylphenyl phosphite (TNPP; CAS # 26523-78-4; from Dover Chemical Corporation): Purity, 99.8%)

Remark: Note: TNPP is known to rapidly hydrolyze in water to NP and phosphorus acid. Therefore, relevant OECD guidelines regarding hydrolytically unstable compounds were employed to conduct aquatic toxicity tests with TNPP. An initial target concentration of 100 mg TNPP/L was used to prepare stock solutions of TNPP's hydrolysis breakdown products. One hundred units of TNPP will yield about 96 units of NP through hydrolysis. Thus, theoretically, 96 mg NP/L could be formed via hydrolysis in the solutions prepared from 100 mg TNPP/L. The theoretical concentration of 96 mg NP/L is well above the measured aqueous solubility of 6 mg/L and above concentrations of NP known to be acutely toxic. However, as noted below, no toxicity was observed in this study that used the stock solution of TNPP breakdown products.

The absence of toxicity to aquatic organisms from solutions of hydrolysis breakdown products is expected based on the known and calculated physical properties of TNPP. TNPP has a calculated octanol water partition coefficient (log₁₀ basis) of 21. This very high log Kow value implies a water solubility well below the estimated aqueous solubility of 0.6 mg/L TNPP. In fact, the aqueous solubility of TNPP may be below 1 microgram per liter, in the nanogram per liter range. Upon entry into water, only very small amounts of TNPP will solubilize in water. It is only dissolved TNPP that will hydrolyze to NP and phosphorus acid. Therefore, if less than 1 microgram of TNPP gets solubilized into one liter of water, less than 1 microgram per liter of NP will be formed. This concentration of NP is well below all available acute toxicity data. Therefore, the absence of toxicity to aquatic organisms was expected using the solutions prepared according to relevant OECD guidelines. Thus, this study should be considered valid without restrictions.

Test conditions: The test was initiated with exponentially growing cells from in-house cultures maintained at 23 ± 2 °C under continuous light (3,770 lux). The culture had a weekly cell yield within 10 % of historical levels. The cultures were grown under axenic conditions in 2-L flasks containing 1 L of artificial media, aerated with filtered sterile air. The composition of the medium is presented below:

Table 4.3.1. Composition of the Growth and Test Media

Nutrient	Concentration (mg/L)	
	Growth media	Test media
NaNO ₃	25.5	15.9
MgCl ₂ -6H ₂ O	10.0	6.25

Nutrient	Concentration (mg/L)	
	Growth media	Test media
CaCl ₂ -2H ₂ O	4.42	2.76
MgSO ₄ -7H ₂ O	14.7	9.19
K ₂ HPO ₄	1.0	0.65
NaHCO ₃	15.0	9.38
H ₃ BO ₃	0.185	0.116
MnCl ₂ -4H ₂ O	0.416	0.160
ZnCl ₂	0.00328	0.00205
CoCl ₂ -6H ₂ O	0.0014	0.00089
CuCl ₂ -2H ₂ O	0.00001	0.000008
Na ₂ MoO ₄ -2H ₂ O	0.00726	0.00454
FeCl ₃ -6H ₂ O	0.16	0.10
Na ₂ EDTA-2H ₂ O	0.30	0.19

Growth inhibition was assessed as the decrease in cell numbers relative to controls. Cell numbers were obtained from optical density measurements at 430 nm calibrated against particle and cell counts at test termination. The dilution water was dechlorinated City of Calgary tap water (charcoal filtered and aerated) spiked with nutrients. The dilution water had a hardness of 198 mg CaCO₃/L, alkalinity of 146 mg CaCO₃/L, pH of 7.6, and conductance of 446 ms/cm. The test solutions were prepared from a stock solution initially containing 100 mg of TNPP in 1 L of dilution water. TNPP is not water-soluble (< 0.6 µg/mL at 24 ± 1 °C). The major hydrolysis product, nonylphenol, is also sparingly soluble in water. However, phosphorous acid released upon hydrolysis of TNPP is water-soluble. The substance was weighed on a glass Petri dish (100 mg) and the dish placed into a 2-L glass, Erlenmeyer flask containing 1 L of dilution water. A magnetic stir bar was added and the mouth of the flask sealed with Parafilm®. The test substance was stirred gently for 78 hours at room temperature (21 ± 2 °C). The test solutions were then prepared from the stock solution of TNPP hydrolysis products as recommended by the OECD for the testing of difficult substances. A 100-mL volume of the hydrolyzed stock solution was poured into a 250-mL plastic container for the highest test concentration (100 mg/L nominal test concentration). A second 100-mL volume of the stock solution was poured into another 250-mL container and serially diluted with 100-mL volumes of dilution water to obtain the remaining test concentrations (50.0, 25.0, 12.5, 6.3, 3.1, and 1.6 mg/L nominal test concentrations). The excess 100-mL volume was discarded. The solutions were spiked with 1 mL of a concentrated nutrient solution and then inoculated (1 mL) to give an initial cell density of 9,664 ± 154 cells/mL. The inoculum was taken from an exponentially growing culture, washed twice with a sodium bicarbonate solution, and the cell number adjusted to give the desired initial cell density in the 100-mL test volume.

Cell counts were done with a Coulter Counter Model ZBI® particle counter equipped with a 100 mm aperture. The solutions were osmotically adjusted prior to counting. The counts at test initiation were done on solutions osmotically adjusted by adding 0.2 mL of a 50 % sodium chloride solution to 20 mL of the inoculated test solution. At test termination, 0.5 mL was removed from six wells (equal volumes from each well) of the control, 12.5 mg/L, and 100.0 mg/L concentrations from each replicate plate. The solutions were osmotically adjusted for counting with the addition of 20 mL of a 0.5 % sodium chloride solution. Each solution was counted until successive counts were within 10 % of each other. The test was conducted in 96-well microplates (Costar®, Corning Incorporated). The plates have 12 columns of 8 wells each. The well volume was 300 µL. The test volume was 150 µL. The outer wells were filled with the control solution. The inner 6 wells in each column were filled with the test solutions. There were three sets of controls on each plate. The pH was measured in one of the control wells and one well of the highest concentration at test initiation and termination with litmus paper (± 0.5 units). The test was conducted in a controlled environment chamber at 23 ± 2 °C under continuous light with an intensity at the plate surface of 4,370 lux provided by cool white fluorescent lights. The plates were read and rotated to a different position under the light bank each day. Optical density measurements at 430 nm were taken at test initiation and at 24, 48, and 72 h with a MRX Microplate Reader (Dynatech Laboratories). Particle counts were made on the controls, the 12.5 mg/L, and 100.0 mg/L test solutions at 72 h. The counts were converted to cell densities with the factor 93.6 (dilution factor of 80 times 1.17, an empirical constant relating instrument counts to cell numbers). A conversion factor was then derived by dividing the cell density by the optical density at 430 nm corrected for the initial optical density reading at test initiation (background). The relationship for converting optical density readings at 430 nm to cell densities was cell density (cells/mL) = 12,845,000 (optical density @ 430 nm at 24, 48, or 72 h minus the reading at test initiation). The optical density readings of the six replicate wells per concentration per plate were averaged and the averages converted into cell densities. The three columns of six control wells on each plate were averaged into a single value for derivation of the toxicity values. The toxicity values for the inhibiting effects of hydrolysis products on growth of *Raphidocelis subcapitata* were derived from the areas under the growth curves. The percent inhibition of cell growth at each test substance concentration was calculated as the difference between the area under the control curve and the area under the growth curve of each test substance concentration. The results from each replicate plate were treated separately for derivation of the toxicity values (three replicates).

Chemical analysis: Two sets of samples were collected for chemical analysis. The first set consisted of samples of the test solutions and control at test initiation. The second set consisted of samples of the test solutions and control incubated under the test conditions for 72 h. The samples of the test solutions and controls were collected

and incubated in 50-mL polypropylene centrifuge tubes. The samples were frozen and stored in darkness at -19 ± 2 °C and then shipped frozen on ice to Reimer Analytical & Associates Inc. (Vancouver, B.C., Canada) for analysis. The test substance was insoluble in water but hydrolyzed on contact with water to form three molecules of nonylphenol and one molecule of phosphorous acid. A 100-mg/L solution of TNPP will yield upon complete hydrolysis 12 mg/L phosphorous and 96 mg/L nonylphenol. The additional mass is a result of hydrolysis (addition of hydrogen and oxygen from water). The samples were not analyzed for TNPP because it is insoluble in water. The samples of the test solutions were analyzed for nonylphenol.

Results: The pH at test initiation and termination in the controls and 100.0 mg/L test solution ranged from 7.0 to 8.0. The initial and final control cell densities were 9,664 cells/mL and 404,000 cells/mL, respectively. This was a 42-fold increase in cell density over the 72-h test period. A 16-fold increase was required for a valid test. The criterion for effect was growth inhibition based on a decrease in the area under the growth curves for each concentration relative to controls. The test medium contains 0.65 mg/L phosphate. Complete hydrolyses of the test substance (100 mg/L) would yield approximately 12 mg/L of phosphorous acid. The cell density in the highest test concentration at 72 h was 344 % greater than the controls. This represents approximately 1.5 additional doublings of the cell population exposed to the hydrolyzed TNPP solution when compared to the controls. The result indicates that hydrolysis of TNPP causes growth stimulation due to the liberation of phosphorous. The LOEC as well as the 24, 48 and 72 h EC50 values were >100 mg/l. The NOEC was the highest concentration tested of 100 mg/l. The level of nonylphenol present in the test solutions under the conditions in which the stock solution was prepared, diluted, and tested was not toxic to unicellular green alga, *Raphidocelis subcapitata*.

Source: TRS Inc. Charlottesville
Reliability: (2) valid with restrictions

(14)

Species: Scenedesmus subspicatus (Algae)
Endpoint: biomass
Exposure period: 72 hour(s)
Unit: mg/l
Analytical monitoring: no
NOEC: = 100 -
EC50: > 100 -

Method: Directive 87/302/EEC, part C, p. 89 "Algal inhibition test"

Year: 1992

GLP: yes

Test substance: TK 10417 (IRGAFOS TNPP), purity >94%

Remark: Nominal test concentrations of 0, 1.23, 3.7, 11, 33 and 100 mg/L were used. The stock solution was prepared by mixing 200 mg of the test substance with 80 mL water and 1 mL of a 0.8% alkylphenol-polyglycol ether and made up to 100 mL

with water. This 100 mL solution was then made up to 1 liter with water.

Each test concentration was tested in 3 replicates. Calculated amounts of the stock solution to produce the desired test concentrations were added to the water.

The algae were then transferred into the flasks. The cell densities were measured at 24, 48, and 72 hour.

The temperature was continuously measured and maintained at 23 +/- 1°C. The pH was measured at 0 and 72 hours and ranged from 7.8 to 8.1.

Result: No significant effects upon biomass were observed at any test concentration.

Test condition: Test concentrations: 0, 1.23, 3.7, 11, 33, 100 mg/l (3 replicates for the test concentrations, 6 replicates for the blank).

Hardness: no information

temp: 23 +/- 1 °C

pH 7.8 - 8.1

Stock solution contained 200 mg/l TNPP

Solvent: alkylphenolpolyglycoether

Initial cell density=9800 cells/mL.

Preculture of algae 3 days under test conditions.

Vessels: 100 mL Erlenmeyer flasks, stoppered with aluminium caps, on Lab-Shaker, 50 mL test solution per flask.

Continuous illumination, cold white fluorescent light, 118 µE/m² sec +/- 20% (approx. 8000 lux.).

Reliability: (2) valid with restrictions

The tested concentrations were probably far above the actual water solubility of the substance.

No analytical follow-up of the test concentrations was performed.

(7)

4.4 Toxicity to Microorganisms e.g. Bacteria

Type: aquatic
Species: activated sludge, domestic
Exposure period: 3 hour(s)
Unit: mg/l
Analytical monitoring: no
IC20: ca. 100 -
IC50: > 100 -

Method: OECD Guide-line 209 "Activated Sludge, Respiration Inhibition Test"

Year: 1988

GLP: no

Test substance: TNPP, no data on purity

Remark: Reference substance: 3,5-dichlorophenol. 32, 10 and 3.2 mg/L.

Deviations from the guideline: instead of a centrifuged sludge, a settled sludge was used. Due to the very low solubility and the expected low toxicity of the substance, only one concentration (100 mg/L) was tested. The test substance was directly added to the test vessel.

Result: Only one concentration was tested (100 mg/l) in duplicates. In one replicate, no inhibition was recorded, in the other, an inhibition of 24% was observed.

Reference substance: IC50 = 16 mg/L

Substance	Conc (mg/L)	Inhibition (%)
Blank 1	0	-3.7
Reference	32	75.3
Reference	10	32.4
Reference	3.2	-6.9
Blank 2	0	3.7
Test Subst.	100	-1.7
Test Subst.	100	24.3

Test condition: Test system: activated sludge from the sewage treatment plant of CH-4153 Reinach on 30/05/88. Sludge concentration = 1.6-1.7 g/L.

Temperature: 20 +/- 2°C

Vessels: 250 mL BOD flasks with gas inlet

Reliability: The test was performed with dechlorinated drinking water.
(3) invalid
25% inhibition were found in a replicate, at 100 mg/L; consequently, a NOEC cannot be determined.

(6)

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Strain: no data
Sex: male/female
No. of Animals: 50
Vehicle: cottonseed oil
Doses: 8.19 - 11.32 - 16.38 - 22.62 - 32.72 g/kg bw.
Value: = 19500 mg/kg bw

GLP: no
Test substance: Tris-nonylphenyl phosphite

Method: Fifty young adult albino rats (5 males and 5 females/group) were fasted for 18 +/- 2 hours and given 5 graded doses (8.19 to 32.72 grams/kg) of TNPP. The sample was prepared as 50% solution in cottonseed oil and doses were administered by stomach tube. Following dosage, the rats

were housed individually and observed for appearance, behavior, body weight and mortality for a 14-day period. A nutritionally adequate diet and water were provided ad libitum. Rats that died as well as survivors sacrificed at the end of the experiment were examined for evidence of gross pathology. The LD50 was computed according to the method of Miller and Tainter.

Remark: All rats showed evidence of abdominal pain and catharsis after dosage. Higher doses resulted in urinary incontinence and prostration. Mortality resulted at scattered intervals over the first five days. Growth of survivors was normal. Gross pathological findings included hemorrhagic lesions in the gastric mucosa and/or duodenum in a few rats that died and hemorrhagic lungs. LD50 calculated to be 19500 +/- 3300 mg/kg.

Test substance: Tri (polynonylphenyl) phosphite/polygard
Purity not specified
Supplier : the Naugatuck Chemical Division of the United States Rubber Company

Reliability: (2) valid with restrictions
The report was scientifically acceptable

(28) (51)

Type: LD50
Species: rat
Strain: Holtzman
Sex: male
No. of Animals: 30
Vehicle: corn oil
Doses: 0.215, 0.464, 1.00, 2.15, 4.64, 10.0 ml/kg bw.
Larger doses could not be administered without exceeding the capacity of the rat stomach
Value: > 10 ml/kg bw

GLP: no
Test substance: Nonylated phenyl phosphite

Method: Samples were administered orally in corn oil (10 or 50% v/v) by stomach tube to six groups composed of five male albino rats at doses of 0.215, 0.464, 1.00, 2.15, 4.64, and 10.0 ml/kg of body weight. Animals were observed for 14 days.

Remark: Sample #1 : there was no mortality at any dosage tested. During the 14-day observation period, the rats in each dosage group exhibited normal appearance and behaviour. The average bodyweight gain for each group of rats was within the normal range values and at gross autopsy, the organs of all animals appeared grossly within normal limits. Oral LD50 of TNPP sample #1 is therefore greater than 10.0 ml/kg bw.
Sample #2 : Oral LD50 of TNPP sample #2 is greater than 10.0 ml/kg bw. No other data related to sample #2 was available.

Test substance: Nonylated phenyl phosphite
2 samples of TNPP (Sample #1 and Sample #2) were tested. Purity not specified but for purposes of this study, the purity of each sample was considered to be 100% and no correction was made for possible impurities.
Supplier : ArgusChemical Corporation

Reliability: (4) not assignable

Very briefly reported study. The report is incomplete : one page gathering the results of sample #2 is missing.

(53)

Type: LD50
Species: rats and mice
Strain: no data
Sex: no data
Vehicle: no data
Doses: 10 g/kg
Value: > 10000 mg/kg bw

Method: A single oral administration of 10 g/kg was given to rats and mice.
GLP: no
Test substance: Triphenyl nonylphosphite

Remark: No mortality was reported. However, a 6-month storage of Polygard TNPP increased toxicity, resulting in mortality of some rats administered 10 g/kg.

Reliability: (4) not assignable
Test report not available.

(25)

Type: LD50
Species: rat
Value: > 5000 mg/kg bw

Method: other
Year: 1991
GLP: no data
Test substance: no data

Source: GREAT LAKES CHEMICAL ITALIA MILAN
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
(29)

Type: LD50
Species: rat
Value: = 10000 mg/kg bw

Method: other
GLP: no data
Test substance: no data

Source: GREAT LAKES CHEMICAL ITALIA MILAN
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
(30)

5.1.2 Acute Inhalation Toxicity

Remark: no data
Source: GREAT LAKES CHEMICAL ITALIA MILAN
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability: (4) not assignable

5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rabbit
Strain: New Zealand white
Sex: male/female
No. of Animals: 10
Vehicle: no vehicle was used
Doses: 2000 mg/kg bw
Value: > 2000 mg/kg bw

Method: OECD Guide-line 402 "Acute dermal Toxicity"
Year: 2001
GLP: yes
Test substance: Tris-nonylphenyl phosphite

Result: Following the 24-hour exposure, all animals (with the exception of one, which weight loss was : 0.02 kg) gained weight during the 14-day post-treatment observation period. No overt signs of systemic toxicity were evident during the course of the study and no animals died. At necropsy, there were no abnormalities or lesions noted. No erythema or edema was observed at any of the test sites. Based on the absence of mortality, the LD50 was determined to be greater than 2000 mg/Kg.

Test condition: Ten rabbits (5/sex), 12 weeks of age and weighing 2.07-2.69 kg, were dosed with a single dermal application of 2000 mg TNPP/kg body weight for 24 hours, using a determined density of 1.1 gm/mL. The test substance was introduced under gauze patches, two single layers thick, and applied directly to the skin of the body surface (approximately 10%) of each of ten animals. Animals were immobilized and the patches were secured in place by wrapping the entire trunk of the animal with an impervious bandage. Test sites were secured to prevent the animals from ingesting the test substance. At the completion of the exposure period, the wrapping was removed and the skin was gently wiped to remove any test substance still remaining. Clinical examination was made at least once a day and gross necropsy was performed on all animals.

Test substance: Tris-nonylphenyl phosphite
Purity not specified
Supplier : Dover Chemical Corporation

Reliability: (1) valid without restriction

(46)

Type: LD50
Species: rat
Strain: Tif:RAI f1
Sex: male/female
No. of Animals: 10
Vehicle: 0.5% (w/v) carboxymethylcellulose in 0.1% (w/v) aqueous polysorbate 80
Doses: 4 ml/kg body weight
Value: > 2000 mg/kg bw

Method: Directive 92/69/EEC, B.3
GLP: yes
Test substance: Tris-nonylphenyl phosphite

Result: Following a 24-hour exposure, all animals gained weight during the 14-day post treatment observation period. Piloerection and hunched posture were seen, being common symptoms in acute dermal tests. The animals recovered within 2 days. No mortality occurred during this study. At necropsy, no deviations from normal morphology were found. Based on the absence of mortality, the LD50 was determined to be greater than 2000 mg/kg.

Test condition: Ten young adult albino rats (5/sex) weighing 225 to 256 g, were dosed with a single dermal application of 2000 mg TNPP/kg body weight for 24 hours. The test substance was evenly dispersed on the skin (area of at least 10% of the body surface, first shaved with an electric clipper) and covered with a gauze-lined semioclusive dressing fastened around the trunk with an adhesive elastic bandage. After 24 hours, the dressing was removed and the skin was cleaned with lukewarm water. Thereafter, the skin reaction was appraised repeatedly. Clinical examination was made once a day and gross necropsy was performed on all animals at the end of the observation period.

Test substance: purity > 94%

Reliability: (1) valid without restriction (20)

Remark: no data

Source: GREAT LAKES CHEMICAL ITALIA MILAN
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

Reliability: (4) not assignable

5.1.4 Acute Toxicity, other Routes

Type: LC50

Species: rat

Strain: Tif:RAIf (SPF)

Sex: male/female

No. of Animals: 10

Vehicle: distilled water containing 0.5% carboxymethylcellulose and 0.1% polysorbate 80.

Doses: one single dose

Route of admin.: i.p.

Value: > 1000 mg/kg bw

GLP: no data

Test substance: Trisnonylphenyl phosphite

Result: Dyspnoea, exophthalmus, ruffled fur and curved body position were seen, being common symptoms in acute tests. Animals recovered within 12 days. No mortality occurred during the study. At autopsy, peritoneal adhesions in the liver and spleen area were found in 8/10 animals.

Test condition: Five male and five female albino rats aged of 7-8 weeks and weighing 195 to 240 g were administered a single dose of 1000 mg/kg bw, by intraperitoneal injection. Animals were weighed at day 1, 7 and 14. Mortality and symptoms were observed daily for 14 days or until all symptoms have disappeared. The animals were submitted to a gross necropsy at the end of the observation period.

Reliability: (2) valid with restrictions

Remark: no data
Source: GREAT LAKES CHEMICAL ITALIA MILAN
 EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability: (4) not assignable

5.2.1 Skin Irritation

Species: rabbit
Exposure: no data
Exposure Time: 4 hour(s)
No. of Animals: 3
Vehicle: no vehicle was used
Result: slightly irritating
EC classificat.: not irritating

Method: OECD Guide-line 404 "Acute Dermal Irritation/Corrosion"
Year: 2001
GLP: yes
Test substance: Tris-nonylphenyl phosphite

Result: All of the animals exhibited a gain in body weight during the study. No overt sign of toxicity was evident in any of the animals during the course of the study. Very slight erythema was observed in all rabbits following the 4-hour exposure. By the 24-hour observation point, the irritation was reversed, with no sign of erythema present at the 24-, 48- and 72-hour observations for all three rabbits. No edema was observed at any of the observation points. Based upon the Draize scale for scoring skin reaction, the test substance is considered a very slight irritant.

Test condition: - Route of administration : A dose of 0.5 ml liquid test substance was applied to small area (approximately 6 cm²) of non abraded skin of 3 rabbits. Each application area was covered with a gauze patch, held in place by a non irritating tape, for an exposure period of 4 hours.
 - Erythema and edema scores were determined for each of three rabbits at 60 min, 24, 48 and 72 hours after patch removal (at minimum).

Test substance: Tris-nonylphenyl phosphite
 Purity :99.3%Supplier : Dover Chemical Corporation
Reliability: (1) valid without restriction

(47)

Species: rabbit
Exposure: Occlusive
Exposure Time: 24 hour(s)
No. of Animals: 6
Vehicle: no data
PDII: 2.5
Result: moderately irritating
EC classificat.: not irritating

Method: based on the proposed guidelines of the USEPA 163.81-5
 "Primary dermal irritation study"
Year: 1978
GLP: no data
Test substance: other

Result: In 3/6 animals, the application sites showed necrosis. In 5/6 animals the erythemas extended beyond the treated areas. Erythema and edema of intact skin were reversed within 7 days, except in abraded skin in 2/6 animals for which erythema was still moderate to severe. The calculated primary irritation index was 2.5 : TNPP was found to cause moderate irritation when applied to intact and abraded rabbit skin.

Test condition: The test was performed on 3 male and 3 female adult New Zealand White rabbits. Gauze patches, laden with 0.5 ml of the test material, were applied to the prepared (entire back and flank electrically shaved) abraded (slightly scarified) and intact skin of the rabbits. After 24h under occlusive conditions the dressings were removed and the skin reaction was appraised upon removal and during an observation period of 7 days.

Reliability: (2) valid with restrictions (43)

Species: rabbit
Result: irritating
EC classificat.: irritating

Method: other
Year: 1981
GLP: no data
Test substance: no data

Remark: Contact dermatitis developed in rabbits after epicutaneous administration of stored Polygard TNPP. The changes healed spontaneously ad integrum within 3 days after drug withdrawal.

Reliability: (4) not assignable
No report available (25)

Species: rabbit
Result: slightly irritating
EC classificat.: not irritating

Method: other
Year: 1993
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Source: GREAT LAKES CHEMICAL ITALIA MILAN
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

Reliability: (4) not assignable (22)

5.2.2 Eye Irritation

Species: rabbit
Concentration: undiluted
Dose: .1 ml
Comment: not rinsed
No. of Animals: 4
Vehicle: none
Result: slightly irritating

EC classificat.: not irritating

Method: OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year: 2001
GLP: yes
Test substance: Tris-nonylphenyl phosphite

Result:

- All of the animals exhibited an increase of body weight during the course of the study.
- No overt sign of toxicity other than the ocular effects was evident during the course of the study in any of the animals.
- Corneal opacity : No corneal opacity was observed in any of the treated eyes at any of the observation periods.
- Iris : the iris response was normal in all treated eyes.
- Conjunctivae : slight conjunctival redness and chemosis were observed in all three treated eyes at the 1-hour observation point. Conjunctival redness and chemosis persisted in 2 of 3 animals for 24 hours and was resolved by the 48 hour time point. In the third animal, all signs of irritation were resolved by the 24-hour observation point.
- No fluorescein staining was observed in any of the treated eyes at all observation points.

Test condition: The initial procedure was performed using a 10% dilution of the substance on one rabbit. As no severe effect was observed, the test was performed using undiluted test substance. 0.1 ml of the test substance was placed in the conjunctival sac of the left eye of each rabbit. The right eye remained untreated and served as the control. Eyes were examined at 1, 24, 48 and 72 hours after treatment. After recording the observations at 24 hours, the eyes of all rabbits were examined with the aid of fluorescein to further characterise corneal opacity. As signs of irritation were present at the 72-hour observation point, scoring was continued to determine the progress of the lesions and the reversibility or irreversibility. The experiment was terminated at that time where reversibility was observed in each individual rabbit and the treated eyes returned to normal, which was before 21 days.

Test substance: Tris-nonylphenyl phosphite
Purity : 99.3%
Supplier : Dover Chemical Corporation

Reliability: (1) valid without restriction (48)

Species: rabbit
Result: slightly irritating
EC classificat.: not irritating

Method: other
Year: 1993
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Source: GREAT LAKES CHEMICAL ITALIA MILAN
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

Reliability: (4) not assignable (22)

Species: rabbit
Dose: .1 ml
Comment: the eyes of the rabbits were rinsed 30 seconds after treatment, the 3 others were not rinsed
No. of Animals: 6
Vehicle: no data
Result: slightly irritating
EC classificat.: not irritating

Method: Based on the proposed guideline of the US EPA "Primary eye irritation study"
Year: 1978
GLP: no data
Test substance: Trisnonylphenyl phosphite

Result: Slight redness and chemosis were the only observable effects. They were completely reversible in rabbits with rinsed eye within 7 days. The test material was found to cause minimal irritation when applied to the rabbit eye mucosa, whether the eyes were rinsed or unrinsed.
Test condition: The test was performed on 3 male and 3 female New Zealand White rabbits. 0.1 ml of the test material was inserted into the conjunctival sac of the left eye of the rabbits. In 3 of the 6 rabbits approximately 30 seconds after treatment, the treated eye was flushed with 10 ml of physiological saline. The eye irritation was appraised with a slit-lamp on day 1, 2, 3, 4 and 7 and was scored for each individual rabbit.
Reliability: (2) valid with restrictions

(42)

5.3 Sensitization

Type: Guinea pig maximization test
Species: guinea pig
Concentration
 1st: Induction 5 % intradermal injection
 2nd: Induction 10 % occlusive epicutaneous
 3rd: Challenge 1 % occlusive epicutaneous
No. of Animals: 30
Vehicle: Oleum arachidis for intradermal induction, Vaseline for epidermal applications
Result: sensitizing
Classification: sensitizing

Method: Directive 84/449/EEC, B.6 "Acute toxicity (skin sensitization)"
Year: 1984
GLP: yes
Test substance: Trisnonylphenyl phosphite

Result: All the animals gained weight during the study. There were 12/20 and 15/20 positive animals respectively 24h and 48h after occlusive epidermal application (showing erythema scores of 1 to 2). Therefore, TNPP is classified as a strong sensitiser in albino guinea pig according to the grading of Magnusson and Kligman.
Test condition: The test was performed on 10 male and 10 female guinea pigs in the test group and 5 male and 5 female in the control

group. Control group : The control group was treated with adjuvant and the vehicle during the induction period. During the challenge period, the control group was treated with the vehicle as well as with the test article to check the maximum subirritant concentration of the test article in adjuvant treated animals.

- Induction (weeks 1 and 2) : it was a 2-stage operation. First, intradermal injection into the neck region (adjuvant/saline mixture, 5% of test article in Oleum arachidis (well tolerated dose) and test article in the adjuvant/saline mixture). Second, one week later, closed patch exposure of 10% TNPP in vaseline (concentration leading to erythema reactions) over the injection sites for 48 hours.
- Rest period : during weeks 3 and 4, no treatment was performed.
- Challenge (week 5) : the animals were tested on the flank with 1% TNPP in vaseline (subirritant concentration) and the vehicle alone (occluded administration for 24 hours). 24 and 48h after removing the dressings, the challenge reactions were graded according to the Draize scoring scale. The body weight was recorded at start and end of the test.

Test substance: purity > 94%
Reliability: (1) valid without restriction

(19)

Type: Buehler Test
Species: guinea pig
Concentration
 1st: Induction undiluted occlusive epicutaneous
 2nd: Challenge undiluted occlusive epicutaneous
No. of Animals: 38
Vehicle: no vehicle
Result: not sensitizing
Classification: not sensitizing

Method: OECD Guide-line 406 "Skin Sensitization"
Year: 2001
GLP: yes
Test substance: Tris-nonylphenyl phosphite

Result:

- During the induction and the challenge phases, all animals gained weight and no abnormal clinical observation was evident in any of the animals during the course of the study.
- All animals showed no sign of erythema or edema at the 24 and 48 hour observation point for the challenge phase. The test substance is not considered to be a skin sensitizer.

Test condition: Induction phase :
0.4 ml of the test substance, undiluted, was applied once per week for 3 consecutive weeks (Day 0,7 and 14) on one side of the animal, directly to the skin and covered with a gauze pad of approximately 4-6 cm². The positive control substance, dinitrochlorobenzene (DNCB), was applied in the same manner. Naive animals (i.e. untreated during the induction phase) served as a negative control.
Challenge phase :
The skin was exposed to the test substance for 6 hours in the same way as for the induction phase. At approximately

21 hours after removal of the challenge dose, the area of the challenge was marked and the whole back shaved. Approximately 3 hours after shaving, the test site was examined for erythema and edema. Reading of the skin area was repeated at approximately 48 hours after removal of the challenge dose and the skin reactions were graded.

Test substance: Tris-nonylphenyl phosphite
Purity : 99.3%
Supplier : Dover Chemical Corporation

Reliability: (1) valid without restriction

(34)

5.4 Repeated Dose Toxicity

Type: Sub-chronic
Species: rat
Sex: male/female
Strain: no data
Route of administration: oral feed
Exposure period: 12 weeks (90 days)
Frequency of treatment: daily
Post exposure period: Body weight and food intake recorded weekly, inspected daily for appearance and behavior.
Doses: TNPP in basal food rations at doses of 0, 0.2, 1.0, and 5.0%
Control Group: yes, concurrent no treatment
NOAEL: = 1 %
LOAEL: = 5 %

Method: see below
Year: 2001
GLP: no
Test substance: Tri (polynonylphenyl) phosphite

Method: Groups of 6 rats/sex were fed TNPP at levels of 0.2, 1.0, and 5.0%. Dietary levels were designed to provide each day the equivalent of 1, 5, and 25% of the acute LD50 (established as approximately 20g/kg). Approximately 48 weanling albino rats (27-29 days of age) were divided into four groups and housed individually in metal cages. Body weight and food intake recorded weekly, inspected daily for appearance and behavior. Hematological and chemical examinations were made on the blood of two male and two female rats per group at the 12-week period. All rats that died and all survivors were examined at autopsy for evidence of gross pathology.

Remark: There were no effects reported at levels up to 1% in the diet. Growth was significantly depressed in both sexes at the 5% level. Two females died in the 5% group (on the 35th and 49th day) and the principle abnormalities seen at necropsy suggested that the deaths were in part due to pulmonary pathology (fibrinous exudate in the thorax and hemorrhagic lungs). Hematological and clinical chemistry examination revealed no effects, even at the highest dietary concentration. The only organ weight measured was the liver and there were no differences noted when compared to the control group. The histopathological examinations disclosed no abnormalities in this organ at any dose level. At necropsy, effects on the kidneys in 8 of 9 animals examined in the 5% group (acute and chronic pyelonephritis

with hydronephrosis) were reported.

Result: Two female rats in the 5% group died, possibly due in part to pulmonary pathology seen commonly in lab rats. Hematological or clinical chemistry parameters were within normal ranges for all treatment groups. At necropsy, the only changes reported were in the kidneys of the 5% treatment group and consisted of acute and chronic pyelonephritis with hydronephrosis.

Test substance: Tri (nonylphenyl) phosphite.
Purity not specified
Supplier : the Naugatuck Chemical Division of the United States Rubber Company

Reliability: (2) valid with restrictions
Non-GLP and did not follow specific international test guidelines. However, it was an acceptable, well-documented study report which meets basic scientific principles.

(51)

Type: Chronic
Species: rat
Sex: male/female
Strain: no data
Route of administration: oral feed
Exposure period: 2 years
Frequency of treatment: daily/ad libitum
Doses: Designed to be equivalent to approximately 1000, 3300, and 10,000 ppm in the diet when the rats reached maturity (0, 50, 167, and 500 mg/kg/day)
Control Group: yes, concurrent no treatment
NOAEL: = 3300 ppm
LOAEL: = 10000 ppm
Method: see below
Year: 1961
GLP: no
Test substance: Tri (polynonylphenyl) phosphite

Method: 200 Weanling rats were distributed into four groups of 25 males and 25 females each. Appearance, behavior, and survival were noted daily. Records of body weight were made weekly for first 12 weeks, and 4-week intervals thereafter. At 12 weeks and at approximately half-yearly intervals, clinical examinations were made in 10 rats of each sex in the control and highest groups and 5 of each sex in the two lower groups. Clinical tests included erythrocyte and leukocyte counts; blood hemoglobin, hematocrit, sugar, and nonprotein nitrogen determinations; and urine protein, sugar, and sediment. Blood cholesterol levels and prothrombin time were made at several intervals. Reproductive studies were initiated after 100 days, and 10 representative rats from each sex were placed on the same ration as the parents. Observations similar to those described above were carried out through these and two additional descendant generations. In the F1 and F2 generations, the animals were carried through only two matings and F3 rats were not mated. Pathology was conducted in F0 rats at the termination of the two-year period, and at various times after the weaning of the second litter. Organ weights included the liver, kidneys, spleen, heart,

gonads, adrenals, thyroid, and pituitary. Histopathological examination was performed on the liver, kidney, spleen, adrenals, thyroids, pituitary, heart, stomach, small and large intestine, pancreas, bladder, gonads, salivary glands, lymph nodes, lungs, bone marrow, muscle, brain and spinal cord. All tumors were examined microscopically.

Remark: Combined chronic and reproductive toxicity study.
Result: Growth was normal at all dose levels in both sexes except for F0 males receiving the highest dose level. The responses of the descendant generations at 12 weeks showed a slight statistically significant retardation in growth of the F2 and F3 males at the 500 mg/kg level. In females, growth was slightly retarded at the highest dose in the F3 generation. The survival data showed few deaths occurring during the first year of the study. Mortality during the second year was significantly higher among the females that were being carried through the reproduction studies than among the males but the effects were not dose-related. No significant treatment-related changes were observed for hematology and clinical chemistry, or anti-cholinesterase activity. There were no significant changes from control animals for any generation with respect to organ weights and dose-related findings at necropsy, except for F0 females receiving 500 mg/kg, where a possibly dose-dependant increase in liver weight was observed. This may be attributable to the stress of frequent pregnancies and lactation with the concomitant increase in food intake and hence, elevation of dosage level. The tumor incidence at all levels of TNPP was approximately the same as that of the control group and consisted mainly of fibroadenomas and fibromas of mammary origin.

Test substance: Tri (polynonylphenyl) phosphite
Purity not specified
Supplier : the Naugatuck Chemical Division of the United States Rubber Company

Reliability: (2) valid with restrictions
Non-GLP and did not follow specific international test guidelines. However, it was an acceptable, well-documented study report which meets basic scientific principles.

(52)

Type: Chronic
Species: dog
Sex: male/female
Strain: no data
Route of administration: oral feed
Exposure period: 2 years
Frequency of treatment: daily/ad libitum
Doses: Dose set at 0, 0.10, 0.33, and 1.0 % (0, 1000, 3300, and 10,000 ppm respectively)
Control Group: yes, concurrent no treatment
NOAEL: = 3300 ppm
LOAEL: = 10000 ppm

Method: see below
GLP: no
Test substance: Tri (polynonylphenyl) phosphite

Method: 24 Beagle dogs were separated into four groups of 3 males

and 3 females each. Appearance, behavior, and survival were noted daily. Records of body weight were made weekly for the first 12 weeks, and at 4-week intervals thereafter. Neurological tests were performed at 12 weeks and frequently thereafter. Clinical tests included erythrocyte and leukocyte counts; blood hemoglobin, hematocrit, sugar and nonprotein nitrogen determinations; and urine protein, sugar, and sediment. Blood cholesterol levels, and prothrombin time were made at several intervals. No reproductive tests were conducted. Organ weights included the liver, kidneys, adrenals, thyroid, and pituitary. Histopathological examination was performed on the liver, kidney, spleen, adrenals, thyroids, pituitary, heart, stomach, small and large intestine, pancreas, bladder, gall bladder, gonads, salivary glands, lymph nodes, lungs, bone marrow, muscle, brain and spinal cord. All tumors were examined microscopically.

Remark: There were no effects reported at concentrations below 10,000 ppm.

Result: All dogs survived the duration of the study with the exception of one female at the low and one female at the middle treatment group after five months. After confirmation of the diagnosis (encephalitic meningitis) at autopsy, these deaths were considered irrelevant to the study and the animals were replaced. No changes in general appearance, body weights, or food consumption were observed. All hematological parameters were within normal ranges throughout the study with the exception of a slight decrease in the hemoglobin and hematocrit levels of the 1% group at 100 weeks. Clinical chemistry parameters were normal with the exception of elevated cholesterol levels in the females of the high treatment group. Neurological parameters measured (patellar, tonic neck and tonic eye reflexes, and placing, supporting and righting reflexes) were normal at all times. The findings at necropsy revealed no changes in organ weights or significant gross abnormalities. Histopathological examination of the thyroid showed slight hyperplastic changes in one control and one high treatment group dog and a moderate degree of hyperplasia of one dog in the high treatment group. These were the only finding of possible significance in the gross histopathological examinations.

Test substance: Tri (polynonylphenyl) phosphite
Purity not specified
Supplier : the Naugatuck Chemical Division of the United States Rubber Company

Reliability: (2) valid with restrictions
Non-GLP and did not follow specific international test guidelines. However, it was an acceptable, well-documented study report which meets basic scientific principles.

(52)

Type: Sub-chronic
Species: rat
Sex: male/female
Strain: Sprague-Dawley
Route of administration: gavage
Exposure period: Males = 4 weeks (2 weeks pre-breeding, 2 weeks mating)
Females = 10 weeks (2 weeks prebreed, 2 weeks mating, 3 weeks gestation, and 3 weeks lactation)

F1 generation dosed until 85 days of age

Frequency of treatment: daily

Post exposure period: 5 F0 males per group from control and 1000 mg/kg/d - dose groups were designated as recovery group and held without dosing for 2 weeks after the F0 male dosing period was completed.

Doses: 0, 50, 200 and 1000 mg/kg/day

Control Group: yes, concurrent vehicle

NOAEL: = 200 mg/kg bw

NOAELfemales: = 1000 mg/kg bw

Year: 2002

GLP: yes

Test substance: Trisnonylphenyl phosphite

Method: OECD Test Guideline 421 (1998). This study exceeds the OECD 421 study design as follows: (1) enhanced evaluation of toxicity in the F0 generation, including the evaluation of a recovery group of F0 males; (2) evaluation of developmental landmarks in the F1 generation; and (3) following the F1 offspring to adulthood, with continued exposure and assessments of reproductive structures and functions, including potential effects on sperm.

Test procedure: Male and female CD® (Sprague-Dawley) rats (the F0 generation) were administered TNPP orally by gavage at 0, 50, 200, or 1000 mg/kg/day at a dose volume of 5 ml/kg/day in Mazola® corn oil, ten/animals/sex/dose, for two weeks of prebreed exposure (males and females) and two weeks of mating (males and females) for F0 parents. F0 females continued to be dosed for three weeks each of gestation and lactation, as were F1 offspring from weaning through scheduled sacrifice, at approximately 85 days of age. Five additional F0 males per group from the control and 1000 mg/kg/day groups were designated as recovery animals and held without dosing for two weeks, after the F0 male dosing period was completed to evaluate recovery from any possible treatment-related effects identified in the high dose. Body weights and feed consumption for the F0 males and females were recorded at least weekly during the prebreed period for both sexes, for F0 females during gestation and lactation, and F1 offspring from birth through scheduled sacrifice. Clinical signs were recorded at least once daily for all animals. After the two-week prebreed exposure period, animals were randomly mated within treatment groups for a two-week mating period to produce the F1 generation, with continuing exposure. On the day of birth (postnatal day [pnd] 0), anogenital distance (AGD) was measured and body weights recorded for all live F1 pups in all litters. F1 litters were culled on pnd 4 to yield, as nearly as possible, five males and five females per litter. The culled F1 pups were weighed, euthanized, and necropsied with complete external and visceral examinations. For the remaining F1 pups, survival indices were calculated at least weekly through weaning (pnd 21). At weaning, at least one female and one male (whenever possible) from each F1 litter were randomly selected to continue treatment for approximately seven more weeks, with dosing for F1 selected pups begun on pnd 22 until all pups were at least 85 days of age. All F0

parental animals, nonselected F1 weanlings, and retained F1 adults were necropsied, with complete histologic evaluation of five selected F0 and F1 males and females in the 0 and 1000 mg/kg/day groups. Histopathology was performed on F1 males and females (five/sex/group) at 0 and 1000 mg/kg/day. The following is a discussion of the F0 and F1 adult systemic toxicity.

Remark:

F0 Adult systemic toxicity : The following discussion focuses on treatment-related effects. Other changes noted in the attached tables were considered random, due to biological variation, and not treatment related. Table 5.4.1 provides a summary of F0 adult systemic toxicity.

Table 5.4.1. Summary of F0 Adult Systemic Toxicity

Trisnonylphenyl Phosphite (mg/kg/day)		F0			
		0	50	200	1000
<u>PARENTAL MALES</u>					
Deaths		0	0	0	0
<u>Prebreed Exposure</u>					
Body Weights		--	--	--	--
Weight Change		--	--	--	↓
Clinical Observations		--	--	--	--
Feed Consumption:					
	g/day	--	↓	--	--
	g/kg/day	--	--	--	↑↑
<u>Necropsy</u>					
Body Weight		--	--	--	--
Organ Weights:					
Liver					
	A	--	--	--	--
	R - Body	--	--	--	--
	R - Brain	--	--	--	--
Paired Kidneys					
	A	--	--	--	↑
	R - Body	--	--	--	↑↑
	R - Brain	--	--	--	↑↑
Brain					
	A	--	--	--	--
	R - Body	--	--	--	--
Thymus					
	A	--	--	--	--
	R - Body	--	--	--	--
	R - Brain	--	--	--	--
Heart					
	A	--	--	--	--
	R - Body	--	--	--	--
	R - Brain	--	--	--	--
Spleen					
	A	--	--	--	--
	R - Body	--	--	--	--
	R - Brain	--	--	--	--
Paired Adrenal Glands					
	A	--	--	--	--
	R - Body	--	--	--	--
	R - Brain	--	--	--	--
Paired Testes					
	A	--	--	--	--
	R - Body	--	--	--	--
	R - Brain	--	--	--	--
Paired Epididymides					
	A	--	--	--	--
	R - Body	--	--	--	--
	R - Brain	--	--	--	--
Prostate					
	A	--	--	--	--
	R - Body	--	--	--	--
	R - Brain	--	--	--	--
Seminal Vesicles					
	A	--	--	--	--
	R - Body	--	--	--	--
	R - Brain	--	--	--	--

Trisnonylphenyl Phosphite (mg/kg/day)		F0			
		0	50	200	1000
Gross Findings		--	--	--	--
Histopathology: ^a					
Kidneys - Corticomedullary junction mineralization					
	Minimal	0			3
<u>F0 FEMALES</u>					
Deaths		0	1	0	4
<u>Prebreed Exposure</u>					
Body Weights		--	--	--	--
Weight Change		--	--	--	--
Clinical Observations		--	--	--	--
Feed Consumption:					
	g/day	--	--	--	↑↑
	g/kg/day	--	--	--	↑↑ ^b
<u>Gestation</u>					
Body Weights		--	--	--	--
Weight Change		--	--	--	--
Clinical Observations		--	--	--	--
Feed Consumption:					
	g/day	--	--	--	↑
	g/kg/day	--	--	--	↑↑
<u>Lactation (pnd 0-21)</u>					
Body Weights		--	--	--	--
Weight Change		--	--	--	--
Clinical Observations		--	--	--	--
Feed Consumption:					
	g/day	--	--	--	--
	g/kg/day	--	--	--	--
<u>Necropsy</u>					
Body Weights		--	--	--	--
Organ Weights:					
Brain	A	--	--	--	--
	R - Body	--	--	--	--
Thymus	A	--	--	--	--
	R - Body	--	--	--	--
	R - Brain	--	--	--	--
Heart	A	--	--	--	--
	R - Body	--	--	--	--
	R - Brain	--	--	--	--
Liver	A	--	--	--	--
	R - Body	--	--	--	--
	R - Brain	--	--	--	--
Spleen	A	--	--	--	--
	R - Body	--	--	--	--
	R - Brain	--	--	--	--
Paired Kidneys	A	--	--	↑	--
	R - Body	--	--	↑	--
	R - Brain	--	--	↑	--
Paired Adrenal Glands	A	--	--	--	--
	R - Body	--	--	--	--
	R - Brain	--	--	--	--
Uterus with Vagina and Cervix	A	--	--	--	--
	R - Body	--	--	--	--
	R - Brain	--	--	--	--
Paired Ovaries	A	--	--	--	↓↓
	R - Body	--	--	--	↓
	R - Brain	--	--	--	↓
Gross Findings		--	--	--	--
Histopathology: ^a					
Kidneys - Corticomedullary junction mineralization					

Trisnonylphenyl Phosphite (mg/kg/day)	F0			
	0	50	200	1000
Minimal	5			1

^a *Histopathology was performed on control and high dose animals only*

↑, ↑↑ = statistically significant increase; one symbol - p<0.05; two symbols - p<0.01

↓, ↓↓ = statistically significant decrease; one symbol - p<0.05; two symbols - p<0.01

^b *Change in one or more intervals*

--- = no statistically significant difference

A = absolute organ weight

R - Body = organ weight relative to terminal body weight (%)

R - Brain = organ weight relative to terminal brain weight (%)

F0 Males: There were no treatment-related deaths for the F0 males. For parental males, minor systemic toxicity was present at 1000 mg/kg/day, expressed as a trend toward decreased body weights and reduced body weight gains. Feed consumption was significantly increased at 1000 mg/kg/day during mating. This finding was considered most likely because of the excessive rooting behavior observed during the dosing period. Paired kidney weights, both absolute and relative to terminal body and brain weight, were significantly increased at 1000 mg/kg/day. There were no treatment-related effects for the gross necropsy findings. However, histological findings included minimal corticomedullary junction mineralization in the kidneys in three of ten males (with no males exhibiting this finding at 0 mg/kg) at 1000 mg/kg/day, which correlated with the increased kidney weights, both absolute and relative to body and brain weight, at this dose. In recovery males, there was a trend toward increasing body weights in the high dose group, so that at the end of the two-week recovery period, the body weights were similar to the control group values. There was no effect on kidney weights in the recovery group.

F0 Females: One F0 female at 50 mg/kg/day and four F0 females at 1000 mg/kg/day were found dead. The unscheduled deaths of the low dose F0 female during gestation and one of the high dose F0 female during lactation were attributed to dosing errors and were not considered treatment related. Of the three remaining unscheduled F0 females deaths, all were found on gestation day 22, possibly attributable to dystocia. Dystocia was evident due to the inability of the dams to deliver their pups. Examination of the pups at maternal necropsy indicated that they were full term and normal in external appearance. F0 parental females did not exhibit any other overt adult systemic toxicity at any dose, as evidenced by a lack of statistically significantly different body weights or weight changes during prebreed, gestation or lactation, changes in feed consumption, or gross necropsy findings. However, trends toward increased feed consumption in females from the high-dose group (except during lactation) were noted. As with the males, because of the clinical signs, particularly of rooting behavior following dosing, the feed consumption differences were considered likely related to this observation. Paired

ovary weights (absolute and relative to terminal body and brain weights) were significantly decreased at 1000 mg/kg/day. Gross necropsy and histological findings of F0 parental females exhibited no treatment- or dose-related pattern of incidence or severity at scheduled sacrifice.

Table 5.4.2. Summary of F1 Adult Systemic Toxicity

Trisnonylphenyl Phosphite (mg/kg/day)		F1			
		0	50	200	1000
<u>F1 ADULT MALES</u>					
Deaths		0	0	0	0
<u>Postweaning Period (pnd 22-85)</u>					
Body Weights		--	--	--	--
Weight Change		--	--	--	--
Clinical Observations		--	--	--	--
Feed Consumption:					
	g/day	--	--	--	↑
	g/kg/day	--	--	--	↑, ↑↑
<u>Necropsy</u>					
Body Weight		--	--	--	--
Organ Weights:					
Liver					
	A	--	--	--	--
	R - Body	--	--	--	--
	R - Brain	--	--	--	--
Paired Kidneys					
	A	--	--	--	--
	R - Body	--	--	--	--
	R - Brain	--	--	--	--
Brain					
	A	--	--	--	--
	R - Body	--	--	--	--
Thymus					
	A	--	--	--	--
	R - Body	--	--	--	--
	R - Brain	--	--	--	--
Heart					
	A	--	--	--	--
	R - Body	--	--	--	--
	R - Brain	--	--	--	--
Spleen					
	A	--	--	--	--
	R - Body	--	--	--	--
	R - Brain	--	--	--	--
Paired Adrenal Glands					
	A	--	--	--	--
	R - Body	--	--	--	--
	R - Brain	--	--	--	--
Paired Testes					
	A	--	--	--	--
	R - Body	--	--	--	--
	R - Brain	--	--	--	--
Paired Epididymides					
	A	--	--	--	--
	R - Body	--	--	--	↓
	R - Brain	--	--	--	--
Prostate					
	A	--	--	--	--
	R - Body	--	--	--	--
	R - Brain	--	--	--	--
Seminal Vesicles					
	A	--	--	--	--
	R - Body	--	--	--	--
	R - Brain	--	--	--	--
Gross Findings		--	--	--	--
Histopathology: ^a					
Kidneys - Corticomedullary junction mineralization					
	Minimal	0			1
	Moderate	0			1
<u>F1 ADULT FEMALES</u>					
Deaths		0	0	0	0

Trisnonylphenyl Phosphite (mg/kg/day)		F1			
		0	50	200	1000
Postweaning Exposure (pnd 22 to 85)					
Body Weights		--	--	--	--
Weight Change		--	--	--	--
Clinical Observations		--	--	--	--
Feed Consumption:	g/day	--	--	--	--
	g/kg/day	--	--	↑	↑
Necropsy					
Body Weights		--	--	--	--
Organ Weights:					
Brain	A	--	--	--	--
	R - Body	--	--	↓	--
Thymus	A	--	--	--	↑↑
	R - Body	--	--	--	--
	R - Brain	--	--	--	↑↑
Heart	A	--	--	--	--
	R - Body	--	--	--	--
	R - Brain	--	--	--	--
Liver	A	--	--	--	--
	R - Body	--	--	--	↑↑↑
	R - Brain	--	--	--	--
Spleen	A	--	--	--	--
	R - Body	--	--	--	--
	R - Brain	--	--	--	--
Paired Kidneys	A	--	--	--	--
	R - Body	--	--	--	--
	R - Brain	--	--	↑↑	↑
Paired Adrenal Glands	A	--	--	--	--
	R - Body	--	--	--	--
	R - Brain	--	--	--	--
Uterus with Vagina and Cervix	A	--	--	--	--
	R - Body	--	--	--	--
	R - Brain	--	--	--	--
Paired Ovaries	A	--	--	--	↓
	R - Body	--	--	--	--
	R - Brain	--	--	--	--
Gross Findings					
Histopathology: ^a					
Kidneys - Corticomedullary junction mineralization	Minimal				3
	Mild				1

^a Histopathology performed on control and high dose animals only

↑, ↑↑, ↑↑↑ = statistically significant increase; one symbol p<0.05; two symbols = p<0.01; three symbols = p<0.001

↓, ↓↓ = statistically significant decrease; one symbol = p<0.05; two symbols = p<0.01

--- = no statistically significant difference

A = absolute organ weight

R - Body = organ weight relative to terminal body weight (%)

R - Brain = organ weight relative to terminal brain weight (%)

F1 Males: There were no unscheduled deaths for the adult F1 males. There were no significant differences in body weight or weight gain for the F1 males during the postweaning period (from pnd 22 to 85) at any dose. Increased feed consumption as g/day and g/kg/day at 1000

mg/kg/day, considered related to increased rooting behavior, was observed. Paired epididymides weights, relative to terminal body weights, were significantly decreased at 1000 mg/kg/day. This finding is of uncertain toxicological significance, since there were no changes in epididymal weight in the F0 generation or in absolute organ weight in the F1 generation. There were no treatment-related effects for gross necropsy findings. Histological findings included corticomedullary junction mineralization in the kidneys of two of ten males (with no males exhibiting this finding at 0 mg/kg/day) at 1000 mg/kg/day and were considered treatment related.

F1 Females: There were no treatment-related deaths for the adult F1 females. There were no significant differences in body weight or weight gain for the F1 females during the postweaning period (from pnd 22 to 85). Feed consumption values (as g/kg/day), presumably associated with excessive rooting behavior, were increased at 1000 mg/kg/day. Also, there were no significant differences in the F1 female estrous cycles across all groups. There were no treatment-related effects for the gross necropsy or histopathological findings.

Result: Summary of Results: Exposure once daily to TNPP at 0, 50, 200, or 1000 mg/kg/day resulted in mild systemic toxicity in F0 parental males at 1000 mg/kg/day, expressed as reduced weight gains, increased kidney weights, and renal histopathologic findings in F0 and F1 males (renal corticomedullary junction mineralization). Three of ten pregnant F0 parental females from the 1000 mg/kg/day group were found dead on Day 22 of gestation. These deaths may have been related to dystocia, since the dams appeared to be unable to deliver their normal appearing pups. In addition, absolute ovarian weights and ovarian weights relative to terminal body or brain weights were reduced in F0 females at 1000 mg/kg/day. These adverse effects observed in dams and linked with reproduction won't be taken into account in this chapter but in chapter 5.8.3. No other consistent, treatment-related effects were observed. NOAEL for males = 200 mg/kg/day and NOAEL for females = 1000 mg/kg/day.

Test substance: Tris-nonylphenyl phosphite
Purity : 99.8%
from Dover Chemical Corporation

Reliability: (1) valid without restriction

(49)

Species: rat
Route of administration: oral feed
Exposure period: 90 days
Frequency of treatment: daily
Doses: 1 and 5 %
NOAEL: = 1 %

Year: 1994
GLP: no data
Test substance: no data

Remark: The substance produced kidney damage at 5% of diet.
Source: GREAT LAKES CHEMICAL ITALIA MILAN
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

Reliability: (4) not assignable

(27)

Species: rat
Route of administration: oral feed
Exposure period: 2 years
Frequency of treatment: daily
Doses: 1 %
NOAEL: = 1 %

Year: 1994
GLP: no data
Test substance: no data

Source: GREAT LAKES CHEMICAL ITALIA MILAN
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
(27)

5.5 Genetic Toxicity 'in Vitro'

Type: Bacterial reverse mutation assay
System of testing: Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and Escherichia coli WP2 uvrA
Concentration: 0, 75, 200, 600, 1800, 5000 ug/plate
Cytotoxic Concentration: With metabolic activation: > 5000 ug/plate
Without metabolic activation: > 5000 ug/plate
Metabolic activation: with and without
Result: negative

Method: OECD Guide-line 471
Year: 2001
GLP: yes
Test substance: Tris-nonylphenyl phosphite

Method: The test system was exposed to the test article via the plate incorporation methodology originally described by Ames et al. (1975) and updated by Maron and Ames (1983). On the day of its use, minimal top agar, containing 0.8 % agar (W/V) and 0.5 % NaCl (W/V), was melted and supplemented with L-histidine, D-biotin and L-tryptophan solution to a final concentration of 50 µM each. Top agar not used with S9 or Sham mix was supplemented with 25 mL of water for each 100 mL of minimal top agar. For the preparation of media and reagents, all references to water imply sterile, deionized water produced by the Milli-Q Reagent Water System. Bottom agar was Vogel-Bonner minimal medium E (Vogel and Bonner, 1956) containing 1.5% (W/V) agar. Nutrient bottom agar was Vogel-Bonner minimal medium E containing 1.5% (W/V) agar and supplemented with 2.5% (W/V) Oxoid Nutrient Broth No. 2 (dry powder). Nutrient Broth was Vogel-Bonner salt solution supplemented with 2.5% (W/V) Oxoid Nutrient Broth No. 2 (dry powder). Each plate was labeled with a code system that identified the test article, test phase, dose level, tester strain, and activation, as described in detail in BioReliance's Standard Operating Procedures. Test article dilutions were prepared immediately before use. One-half (0.5) mL of S9 or Sham mix, 100 µL of tester strain and 50 µL of vehicle or test article dilution were added to 2.0 mL of molten selective top agar at 45±2°C. After vortexing, the mixture

was overlaid onto the surface of 25 mL of minimal bottom agar. When plating the positive controls, the test article aliquot was replaced by a 50 μ L aliquot of appropriate positive control. After the overlay had solidified, the plates were inverted and incubated for approximately 48 to 72 hours at $37 \pm 2^\circ\text{C}$. Plates that were not counted immediately following the incubation period were stored at $2-8^\circ\text{C}$ until colony counting could be conducted. The condition of the bacterial background lawn was evaluated for evidence of test article toxicity by using a dissecting microscope. Precipitate was evaluated by visual examination without magnification. Revertant colonies for a given tester strain and activation condition, except for positive controls, were counted either entirely by automated colony counter or entirely by hand unless the assay was the preliminary toxicity assay or the plate exhibited toxicity. Plates with sufficient test article precipitate to interfere with automated colony counting were counted manually. For each replicate plating, the mean and standard deviation of the number of revertants per plate were calculated and are reported. For the test article to be evaluated positive, it must cause a dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test article. Data sets for tester strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than three times the mean vehicle control value. Data sets for tester strains TA98, TA100 and WP2 uvrA were judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than two times the mean vehicle control value.

Remark:

Criteria for evaluating results: All criteria for a valid test were met. To meet this criteria, all Salmonella tester strain cultures must demonstrate the presence of the deep rough mutation (rfa) and the deletion in the uvrB gene. Cultures of tester strains TA98 and TA100 must demonstrate the presence of the pKM101 plasmid R-factor. All WP2 uvrA cultures must demonstrate the deletion in the uvrA gene. All cultures must demonstrate the characteristic mean number of spontaneous revertants in the vehicle controls as follows (inclusive): TA98, 10-50; TA100, 80-240; TA1535, 5-45; TA1537, 3-21; WP2 uvrA, 10-60. To ensure that appropriate numbers of bacteria are plated, tester strain culture titers must be greater than or equal to 0.3×10^9 cells/mL. The mean of each positive control must exhibit at least a three-fold increase in the number of revertants over the mean value of the respective vehicle control. A minimum of three non-toxic dose levels are required to evaluate assay data. A dose level is considered toxic if one or both of the following criteria are met: (1) A $>50\%$ reduction in the mean number of revertants per plate as compared to the mean vehicle control value. This reduction must be accompanied by an abrupt dose-dependent drop in the revertant count. (2) A reduction in the background lawn. Plates/test: Samples were run in triplicate, with and without metabolic activation. Activation system: Aroclor 1254-induced rat liver S9 was used as the metabolic activation system. The S9 was prepared from male Sprague-Dawley rats induced with a single intraperitoneal injection of Aroclor 1254, 500 mg/kg, five days prior to sacrifice.

Negative and Positive controls: Acetone was the vehicle for the test article and served as the negative control. 2-Aminoanthracene was used for all Salmonella (1 µg per plate) and Escherichia coli (10 µg per plate) with S9 activation. 2-Nitrofluorene (1 µg per plate), sodium azide (1 µg per plate), 9-aminoacridine (75 µg per plate), and methyl methanesulfonate (1,000 µg per plate) were used for strains TA98, TA100/TA1535, TA1537, and WP2 uvrA, respectively.

Result:

The assay was performed in two phases, using the plate incorporation method. The first phase, the preliminary toxicity assay, was used to establish the dose-range for the mutagenicity assay. The second phase, the mutagenicity assay, was used to determine the mutagenic potential of the test article. Acetone was selected as the solvent of choice based on compatibility with the target cells and solubility of the test article. The test article was soluble and clear in acetone at approximately 500 mg/mL, the highest concentration tested. In the preliminary toxicity assay, the maximum dose tested was 5000 µg per plate; this dose was achieved using a concentration of 100 mg/mL and a 50 µL plating aliquot. Precipitate was observed beginning at 1000 µg per plate. No appreciable toxicity was observed. Based on the findings of the toxicity assay, the maximum dose plated in the mutagenicity assay was 5000 µg per plate. In the mutagenicity assay, no positive response was observed. Precipitate was observed beginning at 600 µg per plate. No appreciable toxicity was observed. No positive responses were observed with any of the tester strains in the presence and absence of S9 activation.

Table 5.5.1. Summary of Results

Average Revertants Per Plate ± Standard Deviation					
Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Liver Microsomes: None					
Vehicle	17 ± 3	96 ± 11	13 ± 2	5 ± 3	17 ± 3
75	16 ± 3	99 ± 10	9 ± 4	5 ± 0	15 ± 3
200	18 ± 2	105 ± 16	9 ± 3	5 ± 3	14 ± 2
600	15 ± 3	108 ± 15	11 ± 1	5 ± 1	15 ± 2
1800	15 ± 3	89 ± 18	14 ± 4	6 ± 1	11 ± 2
5000	11 ± 3	95 ± 13	11 ± 2	6 ± 1	13 ± 2
Positive	91 ± 13	332 ± 10	195 ± 7	448 ± 50	77 ± 10
Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Liver Microsomes: Rat Liver S9					
Vehicle	18 ± 4	90 ± 4	8 ± 4	7 ± 1	11 ± 2
75	16 ± 6	88 ± 10	10 ± 1	6 ± 3	11 ± 2
200	16 ± 5	97 ± 7	10 ± 3	6 ± 1	14 ± 2
600	20 ± 1	92 ± 11	10 ± 3	5 ± 3	11 ± 1
1800	15 ± 3	85 ± 7	11 ± 2	5 ± 3	12 ± 1
5000	15 ± 3	84 ± 9	10 ± 2	4 ± 2	10 ± 1
Positive	279 ± 134	356 ± 19	50 ± 8	32 ± 11	86 ± 8

Test substance: Tris-nonylphenyl phosphite, from Dover Chemical Corporation
Purity: 98-99%

Reliability: (1) valid without restriction

(55)

Type: Bacterial reverse mutation assay
System of testing: Salmonella typhimurium strain, TA98, TA100, and TA1535 and TA 1537
Concentration: 313, 625, 1250, 2500 and 5000 µg/0.1ml
Cytotoxic Concentration: no cytotoxic concentration up to 5000 µg/0.1ml
Metabolic activation: with and without
Result: negative

Method: OECD Guide-line 471
Year: 1983
GLP: yes
Test substance: Trisnonylphenyl phosphite

Remark: The test substance is considered to be positive in this test system if a reproducible increase of the mean number of revertants per plate above that of the negative control, at any concentration level, by at least a factor of 1.5 and 2 respectively for strain TA 100 and for strains TA 98, TA 1535 and TA 1537, is observed.

Result: In the experiments performed without and with microsomal activation, comparison of the number of histidine-prototrophic mutants in the controls and after treatment with TNPP revealed no marked differences. At the concentrations of 2500 and 5000 µg/0.1 ml, the test substance precipitated in soft agar. No evidence of the induction of point mutations by TNPP or by its metabolites formed as a result of microsomal activation was detectable in the strains of Salmonella typhimurium used in these experiments.

Test condition: Salmonella typhimurium tester strains TA98, TA100, TA1535 and TA1537 were exposed to TNPP with and without S9 activation. The activation mixture contained S9 fraction of liver from rats induced with Aroclor and a solution of co-factors. The substance was dissolved in acetone. Acetone alone was used for the negative controls. Positive control experiments were carried out simultaneously with the following substances : 1) for strain TA 98 : donorubicin-HCl 2) for strain TA 100 : 4-nitroquinoline-N-oxide 3) for strain TA 1535 : sodium azide 4) for strain TA 1537 : 9(5)-amino-acridine hydrochloride monohydrate. All dose levels of test article, negative controls and positive controls were plated in triplicate and the experiments were repeated in order to confirm the results. A preliminary toxic assay (9 concentrations ranging from 20 to 5000 µg/0.1ml) was used to establish the dose-range over which the test article would be assayed. From the results obtained, the highest concentration suitable for the mutagenicity test was found to be 5000 µg/0.1 ml.

Test substance: Purity > 94%
Reliability: (1) valid without restriction

(11)

Type Bacterial reverse mutation assay
System of testing: Salmonella typhimurium strain TA97, TA98, TA100, and TA102 and Escherichia coli strain WP2/pKM102
Concentration: Up to 5,000 ug/plate
Cytotoxic Concentration: With metabolic activation: Not specified
Without metabolic activation: Not specified
Metabolic activation: with and without
Result: negative

Method: not specified
GLP: no
Test substance: TNPP

Remark: Procedure: Not specified
Plates/Test: Not specified
Activation System: Not specified
Media: Not specified
No. Replicates: Not specified

Reliability: (3) invalid
Not reliable. Documentation is insufficient for assessment.

(18)

Type: Mammalian cell gene mutation assay
System of testing: Mouse lymphoma L5178Y cells
Concentration: 0, 5, 10, 25, 50, 100, and 150 ug/mL
Cytotoxic Concentration: With metabolic activation: >= 150 ug/mL
Without metabolic activation: >= 150 ug/mL
Metabolic activation: with and without
Result: negative

Method: OECD Guide-line 476
Year: 2001
GLP: yes
Test substance: Tris-nonylphenyl phosphite

Method: The preliminary toxicity assay was used to establish the optimal dose levels for the mutagenesis assay. L5178Y cells were exposed to the solvent alone and nine concentrations of test article ranging from 0.5 to 5000 µg/mL in both the absence and presence of S9-activation with a 4-hour exposure and without activation with a 24-hour exposure. Cell population density was determined 24 and 48 hours after the initial exposure to the test article. The cultures were adjusted to 3E05 cells/mL after 24 hours only. Cultures with less than 3E05 cells/mL were not adjusted. Toxicity was measured as suspension growth of the treated cultures relative to the growth of the solvent control cultures. Treatment was carried out in conical tubes by combining 6E06 L5178Y/TK+/- cells, 4 mL FOP medium or S9 activation mixture and 100 mL dosing solution of test or control article in solvent or solvent alone in a total volume of 10 mL. At least eight concentrations of test article were tested in duplicate. The positive controls were treated with MMS (at final concentrations in treatment medium of 10 and 20 mg/mL) and 7,12-DMBA (at final concentrations in treatment medium of 1.0 and 2.5 mg/mL). Treatment tubes were gassed with 5±1% CO₂ in air, capped tightly, and incubated with mechanical mixing for 4 hours at 37±1°C. The preparation and addition of the test article dosing solutions were carried out under amber lighting and the cells were incubated in the dark during the exposure period. After the treatment period, the cells were washed twice with FOP or FOP supplemented with 10% horse serum, 2 mM L-glutamine, 100 U penicillin/mL and 100 µg streptomycin/mL (F10P). After the second wash, the cells were resuspended in F10P, gassed with 5±1% CO₂ in air and placed on the roller drum apparatus at 37±1°C.

Remark: Expression of the mutant phenotype: The cultures were counted using an electronic cell counter and adjusted to

3E05 cells/mL at approximately 24 and 48 hours after treatment in 20 and 10 mL total volume, respectively. Cultures with less than 3E05 cells/mL were not adjusted. For expression of the TK^{-/-} cells, cells were placed in cloning medium (C.M.) containing 0.23% dissolved granulated agar in FOP plus 20% horse serum. Two flasks per culture to be cloned were labeled with the test article concentration, activation condition, and either TFT (trifluorothymidine, the selective agent) or VC (viable count). Each flask was prewarmed to 37±1°C, filled with 100 mL C.M., and placed in an incubator shaker at 37±1°C until used. The cells were centrifuged at 1000 rpm for 10 minutes and the supernatant was decanted. The cells were then diluted in C.M. to concentrations of 3E06 cells/100 mL C.M. for the TFT flask and 600 cells/100 mL C.M. for the VC flask. After the dilution, 1.0 mL of stock solution of TFT was added to the TFT flask (final concentration of 3 mg/mL) and both this flask and the VC flask were placed on the shaker at 125 rpm and 37±1°C. After 15 minutes, the flasks were removed and 33 mL of the cell suspension was pipetted into each of three appropriately labeled petri dishes. To accelerate the gelling process, the plates were placed in cold storage (approximately 4°C) for approximately 30 minutes. The plates were then incubated at 37±1°C in a humidified 5±1% CO₂ atmosphere for 10-14 days. Scoring procedures: After the incubation period, the VC plates were counted for the total number of colonies per plate and the total relative growth determined. The TFT-resistant colonies were then counted for each culture with =10% total relative growth. The diameters of the TFT-resistant colonies for the positive and solvent controls and, in the case of a positive response, the test article-treated cultures were determined over a range of approximately 0.2 to 1.1 mm. Evaluation of results: The cytotoxic effects of each treatment condition were expressed relative to the solvent-treated control for suspension growth over two days post-treatment and for total growth (suspension growth corrected for plating efficiency at the time of selection). The mutant frequency (number of mutants per 1E06 surviving cells) was determined by dividing the average number of colonies in the three TFT plates by the average number of colonies in the three corresponding VC plates and multiplying by the dilution factor (2E-04) then multiplying by 1E06. In evaluation of the data, increases in mutant frequencies that occurred only at highly toxic concentrations (i.e., less than 10% total growth) were not considered biologically relevant. All conclusions were based on sound scientific judgment; however, the following criteria are presented as a guide to interpretation of the data (1) A result was considered positive if a concentration-related increase in mutant frequency was observed and one or more dose levels with 10% or greater total growth exhibited mutant frequencies of =100 mutants per 1E06 clonable cells over the background level. (2) A result was considered equivocal if the mutant frequency in treated cultures was between 55 and 99 mutants per 1E06 clonable cells over the background level. (3) A result was considered negative if the mutant frequency in treated cultures was fewer than 55 mutants per 1E06 clonable cells over the background level. Criteria for evaluating results: For the negative control, the spontaneous mutant frequency

of the cultures must be within 20 to 100 TFT-resistant mutants per 1E06 surviving cells. The cloning efficiency of the solvent control group must be greater than 50%. For positive controls, at least one concentration of each positive control must exhibit mutant frequencies of =100 mutants per 1E06 clonable cells over the background level. The colony size distribution for the MMS positive control must show an increase in both small and large colonies. For the TNPP cultures, a minimum of four analyzable concentrations with mutant frequency data was required. Plates/test: Samples were run in duplicate, with and without metabolic activation. Activation system: Aroclor 1254-induced rat liver S9 was used as the metabolic activation system. The S9 was prepared from male Sprague-Dawley rats induced with a single intraperitoneal injection of Aroclor 1254, 500 mg/kg, five days prior to sacrifice. Negative and Positive controls: Acetone was the vehicle for the test article and served as the negative control. TNPP was soluble in acetone at concentrations up to 500 mg/mL. Methyl methanesulfonate (MMS) was used as the positive control for the non-activated test system at a stock concentration of 1000 and 2000 mg/mL 7,12-Dimethylbenz(a)anthracene (7,12-DMBA) was used as the positive control for the S9-activated test system at stock concentrations of 100 and 250 mg/mL.

Result:

The maximum dose tested in the preliminary toxicity assay was 5000 µg/mL. Visible precipitate was present at concentrations of >=150 µg/mL in treatment medium. No visible precipitate was present at concentrations of >=50 µg/mL in treatment medium. The osmolality of the solvent control was 308 mmol/kg and the osmolality of the highest soluble dose, 50 µg/mL, was 308 mmol/kg. Suspension growth relative to the solvent controls was 23% without activation and 94% with S9 activation at 5000 µg/mL. Based on the results of the toxicity test, the doses chosen for the mutagenesis assay ranged from 1.0 to 200 µg/mL for both the non-activated and S9-activated cultures. Visible precipitate was present at concentrations >=100 µg/mL in treatment medium. No visible precipitate was present at concentrations of <=50 µg/mL in treatment medium. In the non-activated system, cultures treated with concentrations of 5.0, 10, 25, 50 and 100 µg/mL were cloned and produced a range in suspension growth of 88% to 106%. In the S9-activated system, cultures treated with the same concentrations were cloned and produced a range in suspension growth of 76% to 94%. No cloned cultures exhibited mutant frequencies that were at least 55 mutants per 10⁶ clonable cells over that of the solvent control. A dose-response trend was not observed in the non-activated or S9-activated systems. The total growths ranged from 75% to 102% for the non-activated cultures at concentrations of 5.0 to 100 µg/mL and 65% to 102% for the S9-activated cultures at concentrations of 5.0 to 100 µg/mL.

Table 5.5.2. Summary of Cloning Data for L5178Y/TK^{+/-} Mouse Lymphoma Cells Without Metabolic Activation (4-hour exposure)

Test Article Concentration (µg/mL)	TFT Colonies		VC Colonies		Mutant Freq. ^a	Induced Mutant Freq. ^b	% Total Growth ^c
	Counts	Mean	Counts	Mean			
Solvent 1 (Acetone)	40 35 50 42 ±6		148 145 145 146 ±1		57		
Solvent 2 (Acetone)	57 58 55 57 ±1		164 162 195 174 ±15		65		
	Mean Solvent Mutant Frequency =				61		
5 A	52 32 58 47 ±11		137 103 107 116 ±15		82	21	75
5 B	39 44 59 47 ±8		155 159 151 155 ±3		61	0	101
10 A	50 61 47 53 ±6		162 123 137 141 ±16		75	14	84
10 B	35 48 52 45 ±7		114 170 111 132 ±27		68	7	87
25 A	58 42 34 45 ±10		140 169 148 152 ±12		59	-3	92
25 B	42 61 38 47 ±10		118 126 131 125 ±5		75	14	80
50 A	38 24 41 34 ±7		165 130 119 138 ±20		50	-11	84
50 B	38 35 42 38 ±3		152 169 148 156 ±9		49	-12	102
100* A	51 61 55 56 ±4		186 144 151 160 ±18		69	8	89
100* B	48 50 40 46 ±4		130 122 145 132 ±10		70	8	83
Positive Control - Methyl Methanesulfonate (µg/mL)							
10	236 184 214 211 ±21		104 120 137 120 ±13		351	290	54
20	145 178 176 166 ±15		39 31 31 34 ±4		988	927	9
* - Precipitating dose level							
^a - Mutant frequency (per 10 ⁶ surviving cells) = (Average # TFT colonies / average # VC colonies) x 200							
^b - Induced mutant frequency (per 10 ⁶ surviving cells) = mutant frequency - average mutant frequency of acetone							
^c - % total growth = (% suspension growth x % cloning growth) / 100							

Table 5.5.3. Summary Cloning Data for L5178Y/TK^{+/-} Mouse Lymphoma Cells With Metabolic Activation (S9) (4-hour exposure)

Test Article Concentration (µg/mL)	TFT Colonies		VC Colonies		Mutant Freq. ^a	Induced Mutant Freq. ^b	% Total Growth ^c
	Counts	Mean	Counts	Mean			
Solvent 1 (Acetone)	51 66 54 57 ±6		112 91 111 105 ±10		109		
Solvent 2	70 55 71 65 ±7		183 188 209 193 ±11		68		
	Mean Solvent Mutant Frequency =				88		
5 A	71 57 58 62 ±6		+ 143 143 143 ±0		87	-2	90
5 B	27 47 + 37 ±8		176 127 183 162 ±25		46	-43	102
10 A	55 55 61 57 ±3		144 149 157 150 ±5		76	-12	93
10 B	64 51 47 54 ±7		151 169 + 160 ±7		68	-21	98
25 A	50 76 70 65 ±11		178 137 170 162 ±18		81	-7	96
25 B	65 74 73 71 ±4		163 145 164 157 ±9		90	2	95
50 A	55 89 67 70 ±14		+ 130 + 130 ±0		108	20	73
50 B	73 64 86 74 ±9		170 157 155 161 ±7		93	4	88
100* A	55 47 58 53 ±5		137 132 105 125 ±14		86	-3	75
100* B	31 25 22 26 ±4		136 106 143 128 ±16		41	-48	65
Positive Control - 7,12 Dimethylbenz(a)anthracene (µg/mL)							
1	188 163 188 180 ±12		40 31 34 35 ±4		1027	938	3
2.5	++		++				
* - Precipitating dose level							
+ - Culture lost							
++ - Too toxic to clone							
^a - Mutant frequency (per 10 ⁶ surviving cells) = (Average # TFT colonies / average # VC colonies) x 200							
^b - Induced mutant frequency (per 10 ⁶ surviving cells) = mutant frequency - average mutant frequency of acetone							

Test substance: Tris-nonylphenyl phosphite, from Dover Chemical Corporation

Purity: 98-99%

Conclusion: All criteria for a valid study were met as described above. The results of the L5178Y/TK+/- Mouse Lymphoma Mutagenesis Assay indicate that, under the conditions of this study, TNPP was concluded to be negative with and without S9 activation.

Reliability: (1) valid without restriction

(41)

Type: Mammalian cell gene mutation assay
System of testing: Chinese hamster cells V79
Concentration: Range with activation : 0.6-16.0 µg/ml
Range without activation : 0.3-8.0 µg/ml
Cytotoxic Concentration: With activation : above 16.0 µg/ml
Without activation : above 8.0 µg/ml
Metabolic activation: with and without
Result: negative

Method: OECD Guide-line 476
Year: 1984
GLP: yes
Test substance: Trisnonylphenyl phosphite

Remark: Criteria for a negative response :
The test substance will be considered to be inactive in this test system :
- if there is no concentration-dependency of the mutant frequency values determined and the highest mutant frequency of a treated culture exceeds that of the solvent controls by a factor lower than 3.0 or the absolute number of clones in the treated and untreated culture with the highest mutant-frequency value differs by less than 20 clones per 10exp6 cells plated. Or
- if there is a concentration-dependancy of the mutant frequency values determined and the mutant frequency in a treated culture exceeds that of the solvent controls only by a factor lower tan 2.5.

Result: In both investigations with and without microsomal activation, criteria for a negative response were reached : a difference in the treated and untreated dishes of at least 20 clones per 106 cells plated was not detected and there was no indication of a concentration mutant-frequency relation in any experiment.

Test condition: TNPP was dissolved in ethanol at room temperature. The study was conducted with and without activation (Aroclor-induced rat liver S9). The cells were treated in the experiments with microsomal activation for 5 hours and in the experiments without microsomal activation for 21 hours. Two negative controls (ethanol) and one positive control (N-nitroso-dimethylamine) were also tested. The results of each original experiment were confirmed in a second and independent experiment (confirmatory experiment). Based on the results of a preliminary toxicity assay, the original experiments were performed at the following concentrations with microsomal activation : 0.6, 1.2, 2.4, 4.8, 7.2, 9.6 and 12.0 µg/ml and without microsomal activation : 0.3, 0.6, 1.2, 2.4, 3.6, 4.8 and 6.0 µg/ml. Because the intended toxicity was not obtained in the original experiments, in the confirmatory experiments, the concentrations applied were increased to 0.8, 1.6, 3.2, 6.4, 9.6, 12.8 and 16.0

µg/ml with microsomal activation and to 0.4, 0.8, 1.6, 3.2, 4.8, 6.4 and 8.0 µg/ml without microsomal activation.

Test substance: Purity > 94%
Reliability: (1) valid without restriction

(12)

Type: Chromosomal aberration test
System of testing: Chinese hamster ovary (CHO) cells
Concentration: 0, 18.75, 37.5, 75, 150, 200 µg/mL (4-hr treatment w/o S9 mix)
0, 6.25, 12.5, 25, 50, 150 (20-hr treatment w/o S9 mix)
0, 18.75, 37.5, 75, 150, 200 (4-hr treatment with S9 mix)

Cytotoxic Concentration: With metabolic activation: > 200 µg/mL
Without metabolic activation: > 200 µg/mL

Metabolic activation: with and without

Result: negative

Method: OECD Guide-line 473
Year: 2001
GLP: yes
Test substance: Tris-nonylphenyl phosphite

Method: The chromosome aberration assay was performed by exposing duplicate cultures of CHO cells to the test article as well as positive and solvent controls. For the chromosome aberration assay, CHO cells were seeded at approximately 5E05 cells/25 cm² flask and were incubated at 37±1°C in a humidified atmosphere of 5±1% CO₂ in air for 16-24 hours. Treatment was carried out by refeeding duplicate flasks with 5 mL complete medium (McCoy's 5A medium supplemented with 10% FBS, 100 units penicillin and 100 µg streptomycin/mL, and 2 mM L-glutamine) for the non-activated study or 5 mL S9 reaction mixture for the S9 activated study, to which was added 50 µL of dosing solution of test or control article in solvent or solvent alone. The osmolality of the highest concentration of dosing solution in the treatment medium was measured. The pH of the highest concentration of dosing solution in the treatment medium was measured using test tape. In the non-activated study, the cells were exposed to the test article for 4 hours or continuously for 20 hours up to the cell harvest at 37±1°C in a humidified atmosphere of 5±1% CO₂ in air. In the 4 hour exposure group, after the exposure period, the treatment medium was removed, the cells washed with CMF-PBS, refed with complete medium and returned to the incubator. Two hours prior to the scheduled cell harvest, Colcemid® was added to duplicate flasks for each treatment condition at a final concentration of 0.1 µg/mL and the flasks returned to the incubator until cell collection. In the S9 activated study, the cells were exposed for 4 hours at 37±1°C in a humidified atmosphere of 5±1% CO₂ in air. After the exposure period, the treatment medium was removed, the cells washed with CMF-PBS, refed with complete medium and returned to the incubator. Two hours prior to the scheduled cell harvest, Colcemid® was added to duplicate flasks for each treatment condition at a final concentration of 0.1 µg/mL and the flasks were returned to the incubator until cell collection. A

concurrent toxicity test was conducted in both the non-activated and the S9 activated test systems. After cell harvest an aliquot of the cell suspension was removed from each culture and counted using a Coulter counter. The presence of test article precipitate was assessed using the unaided eye. Cell viability was determined by trypan blue dye exclusion. The cell counts and percent viability were used to determine cell growth inhibition relative to the solvent control. Two hours after the addition of Colcemid®, metaphase cells were harvested for both the non-activated and S9 activated studies by trypsinization. Cells were collected approximately 20 hours after initiation of treatment. The cells were collected by centrifugation at approximately 800 rpm for 5 minutes. The cell pellet was resuspended in 2-4 mL 0.075 M potassium chloride (KCl) and allowed to stand at room temperature for 4-8 minutes. The cells were collected by centrifugation, the supernatant aspirated and the cells fixed with two washes of approximately 2 mL Carnoy's fixative (methanol:glacial acetic acid, 3:1, v/v). The cells were stored overnight or longer in fixative at approximately 2-8°C. To prepare slides, the fixed cells were centrifuged at approximately 800 rpm for 5 minutes, the supernatant was aspirated, and 1 mL fresh fixative was added. After additional centrifugation (at approximately 800 rpm for 5 minutes) the supernatant fluid was decanted and the cells resuspended to opalescence in fresh fixative. A sufficient amount of cell suspension was dropped onto the center of a glass slide and allowed to air dry. Slides were identified by the BioReliance study number, date prepared and the treatment condition. The dried slides were stained with 5% Giemsa, air dried and permanently mounted. In the absence of at least 50% toxicity at any dose level, the selection of dose levels for analysis of chromosome aberrations in CHO cells was based upon precipitation of the test article in treatment medium. The highest dose level evaluated was the lowest precipitating dose level, with a sufficient number of scorable metaphase cells. At least two additional lower dose levels were included in the evaluation.

Remark:

Evaluation of metaphase cells: Slides were coded using random numbers by an individual not involved with the scoring process. To ensure that a sufficient number of metaphase cells were present on the slides, the percentage of cells in mitosis per 500 cells scored (mitotic index) was determined for each treatment group. Metaphase cells with 20±2 centromeres were examined under oil immersion without prior knowledge of treatment groups. Initially, the non-activated and S9 activated 4 hour exposure groups were evaluated for chromosome aberrations and if a positive result was obtained in the non-activated 4 hour exposure group, the non-activated 20 hour continuous exposure group was not necessarily evaluated for chromosome aberrations.

Whenever possible, a minimum of 200 metaphase spreads (100 per duplicate flask) were examined and scored for chromatid-type and chromosome-type aberrations. The number of metaphase spreads that were examined and scored per duplicate flask may have been reduced if the percentage of aberrant cells reaches a statistically significant level before 100 cells are scored. Chromatid-type aberrations include chromatid and isochromatid breaks and exchange

figures such as quadriradials (symmetrical and asymmetrical interchanges), triradials, and complex rearrangements. Chromosome-type aberrations include chromosome breaks and exchange figures such as dicentrics and rings. Fragments (chromatid or acentric) observed in the absence of any exchange figure were scored as a break (chromatid or chromosome). Fragments observed with an exchange figure were not scored as an aberration but instead were considered part of the incomplete exchange. Pulverized chromosome(s), pulverized cells and severely damaged cells (=10 aberrations) were also recorded. Chromatid and isochromatid gaps were recorded but not included in the analysis. The XY coordinates for each cell with chromosomal aberrations were recorded using a calibrated microscope stage. Polyploid and endoreduplicated cells were evaluated from each treatment flask per 100 metaphase cells scored. Evaluation of test results: The toxic effects of treatment were based upon cell growth inhibition relative to the solvent-treated control and are presented for the toxicity and aberration studies. The number and types of aberrations found, the percentage of structurally and numerically damaged cells (percent aberrant cells) in the total population of cells examined, and the mean aberrations per cell were calculated and reported for each group. Chromatid and isochromatid gaps are presented in the data but are not included in the total percentage of cells with one or more aberrations or in the frequency of structural aberrations per cell. Statistical analysis of the percent aberrant cells was performed using the Fisher's exact test. Fisher's test was used to compare pairwise the percent aberrant cells of each treatment group with that of the solvent control. In the event of a positive Fisher's test at any test article dose level, the Cochran-Armitage test was used to measure dose-responsiveness. All conclusions were based on sound scientific basis; however, as a guide to interpretation of the data, the test article was considered to induce a positive response when the percentage of cells with aberrations is increased in a dose-responsive manner with one or more concentrations being statistically significant ($p \leq 0.05$). Test articles not demonstrating a statistically significant increase in aberrations will be concluded to be negative. Criteria for evaluating results: The frequency of cells with structural chromosome aberrations in the solvent control must be within the range of the historical solvent control. The percentage of cells with chromosome aberrations in the positive control must be statistically increased ($p \leq 0.05$, Fisher's exact test) relative to the solvent control. Plates/test: Samples were run in duplicate, with and without metabolic activation. Activation system: Aroclor 1254-induced rat liver S9 was used as the metabolic activation system. The S9 was prepared from male Sprague-Dawley rats induced with a single intraperitoneal injection of Aroclor 1254, 500 mg/kg, five days prior to sacrifice. Negative and Positive controls: Mitomycin C (MMC) was used as the positive control in the non-activated study at final concentrations of 0.1 and 0.2 $\mu\text{g/mL}$. Cyclophosphamide (CP) was used as the positive control in the S9 activated study at a final concentration of 10 $\mu\text{g/mL}$. The solvent vehicle for the test article (Acetone) was used as the solvent control at the same concentration as that found in the test

Result:

article-treated groups.

The osmolalities in (I) the treatment medium of the solvent (acetone), (II) the highest concentration tested (200 µg/mL), (III) the lowest precipitating dose in the absence of S9 (150 µg/mL), and (IV) the highest soluble dose in the non-activated 20 hour continuous exposure group (50 µg/mL) were 292, 290 294, and 302 mmol/kg, respectively. The pH of the highest concentration of test article in treatment medium was approximately 7. 4-hr harvest without metabolic activation: No toxicity (cell growth inhibition relative to the solvent control) was observed with TNPP in CHO cells when treated for 4 hours in the absence of S9. The mitotic index at the highest dose level evaluated for chromosome aberrations, 150 µg/mL, was 24% reduced relative to the solvent control. The dose levels selected for microscopic analysis were 37.5, 75, and 150 µg/mL. The percentage of cells with structural aberrations in the test article-treated groups was not significantly increased above that of the solvent control ($p > 0.05$, Fisher's exact test). The percentage of cells with numerical aberrations in the test article-treated groups was significantly increased above that of the solvent control at dose level 150 µg/mL ($p = 0.01$, Fisher's exact test). The Cochran-Armitage test was also positive for a dose response ($p = 0.05$). However, the percentage of cells with numerical aberrations (5.5%) was within the historical solvent control range of 0% to 8.0%. Therefore, it is not considered biologically significant. The percentage of structurally damaged cells in the MMC group was found to be statistically significant (27.0%). 4-hr harvest with metabolic activation: No toxicity (cell growth inhibition relative to the solvent control) was observed with TNPP in CHO cells when treated for 4 hours in the presence of S9. The mitotic index at the highest dose level evaluated for chromosome aberrations, 75 µg/mL, was 12% reduced relative to the solvent control. The dose levels selected for microscopic analysis were 18.75, 37.5, and 75 µg/mL. The percentage of cells with structural or numerical aberrations in the test article-treated groups was not statistically increased above that of the solvent control ($p > 0.05$, Fisher's exact test). The percentage of structurally damaged cells in the CP group was found to be statistically significant (20.0%). 20-hr harvest without metabolic activation: In the absence of a positive response in the non-activated 4 hour exposure group, slides from the non-activated 20 hour exposure group were evaluated for chromosome aberrations. No toxicity (cell growth inhibition relative to the solvent control) was observed with TNPP. The mitotic index at the highest dose level evaluated for chromosome aberrations, 150 µg/mL, was 10% reduced relative to the solvent control. The dose levels selected for microscopic analysis were 25, 50, and 150 µg/mL. The percentage of cells with structural or numerical aberrations in the test article-treated groups was not significantly increased above that of the solvent control ($p > 0.05$, Fisher's exact test). The percentage of structurally damaged cells in the MMC group was found to be statistically significant (25.0%). See Table 5.5.1 (Summary of Test Results).

Table 5.5.5. Summary of Test Results

Treatment (µg/mL)	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored	Aberrations Per Cell (Mean +/- SD)	Cells With Numerical (%)	Aberrations Structural (%)
Vehicle (Acetone)	-	4	11.5	200	0.035 ±0.184	1.5	3.5
Phenol, nonyl-, phosphite (3:1)							
37.5	-	4	9.9	200	0.025 ±0.186	4.0	2.0
75	-	4	10.9	200	0.060 ±0.342	4.0	4.0
150	-	4	8.7	200	0.015 ±0.122	5.5**	1.5
Positive control (MMC) 0.2	-	4	11.5	100	0.370 ±0.691	3.0	27.0**
Acetone	+	4	12.6	200	0.030 ±0.171	2.5	3.0
Phenol, nonyl-, phosphite (3:1)							
18.75	+	4	13.1	200	0.035 ±0.184	3.0	3.5
37.5	+	4	10.2	200	0.045 ±0.231	3.0	4.0
75	+	4	11.1	200	0.055 ±0.250	1.5	5.0
Positive control (CP) 10	+	4	7.8	200	0.230 ±0.498	0.5	20.0**
Vehicle (Acetone)	-	20	10.7	200	0.030 ±0.171	2.5	3.0
Phenol, nonyl-, phosphite (3:1)							
25	-	20	10.8	200	0.065 ±0.302	4.5	5.5
50	-	20	8.9	200	0.040 ±0.221	6.0	3.5
150	-	20	9.6	200	0.065 ±0.267	5.5	6.0
Positive control (MMC) 0.1	-	20	9.2	100	0.320 ±0.649	5.0	25.0**

Treatment: Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.

Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.

Percent Aberrant Cells: *, p<0.05; **, p<0.01; using the Fisher's exact test.

Test substance: Tris-nonylphenyl phosphite, from Dover Chemical Corporation
Purity: 98-99%

Conclusion: The positive and solvent controls fulfilled the requirements for a valid test. Under the conditions of the assay described in this report, TNPP was concluded to be negative for the induction of structural and numerical chromosome aberrations in Chinese hamster ovary cells.

Reliability: (1) valid without restriction

(13)

Type:
System of testing: Chinese ovary cell line CCL 61
Concentration: 65.5, 125.0 and 250.0 µg/ml (original studies)
31.25, 65.5 and 125.0 µg/ml (confirmatory studies)
Cytotoxic Concentration: 28.8 to 47.3% suppression of mitotic activity at
250.0 µg/ml and no viable cells found at 500 µg/ml
Metabolic activation: with and without
Result: negative

Method: OECD Guide-line 473
Year: 1983
GLP: yes
Test substance: Trisnonylphenyl phosphite

Remark: Based on the results of a preliminary toxicity assay, the 2 experiments of the original study were performed at the following concentrations : 62.5, 125.0 and 250.0 µg/ml (with activation and without activation). Since at the upper concentration of 250.0 µg/ml, only metaphase of inferior quality, insufficient for scoring were present, the concentrations of 31.25, 62.5 and 125.0 µg/ml were selected for the four experiments of the confirmatory study (with and without activation).

Criteria for a positive response :

- A test substance is considered to be active in this test system if in comparison to the negative control a marked increase in the number of specific chromosomal aberrations appears or if an increased number of exchange figures appears together with a high number of other specific chromosomal aberrations such as breaks and fragments.
- A concentration-related response in the number of aberrations should be demonstrable.

Result: The number of cells with specific chromosomal aberrations in the treatment groups showed no marked difference in comparison with the negative control. The incidence of changes observed is within the range of spontaneous aberrations inherent to this particular cell line used.

Test condition: TNPP was dissolved in acetone. Acetone was used as negative control. Mitomycin C 0.2µg/ml, a mutagen not requiring S9 activation and cyclophosphamide 40.0 µg/ml, which requires activation, were used as positive controls. The cytotoxicity test was performed as an integral part of the mutagenicity test. Concentrations ranged from 3.91 to 500.0 µg/ml. In the studies for chromosome analysis, the test conditions were the following :

Original study Experiment 1 : 62.5-250.0 µg/ml, in test without activation.

Duration of treatment : 18 hours. Recovery : 0 hours.

Experiment 2 : 62.5-250.0 µg/ml, in test with activation.

Duration of treatment : 3 hours. Recovery : 15 hours.

Confirmatory studies :

Experiment 1 : 31.25-125.0 µg/ml, in test without activation.

Duration of treatment : 18 hours. Recovery : 0 hours.

Experiment 2 : 31.25-125.0 µg/ml, in test with activation.

Duration of treatment : 3 hours. Recovery : 15 hours.

Experiment 3 : 31.25-125.0 µg/ml, in test without activation.

Duration of treatment : 42 hours. Recovery : 0 hours.

Experiment 4 : 31.25-125.0 µg/ml, in test with activation.

Duration of treatment : 3 hours. Recovery : 39 hours.

Reliability: (1) valid without restriction

(45)

5.8.2 Developmental Toxicity/Teratogenicity

Species: rat
Strain: Sprague-Dawley
Route of administration: gavage
Exposure period: Males = 4 weeks (2 weeks pre-breeding, 2 weeks mating)
Females = 10 weeks (2 weeks prebeed, 2 weeks mating, 3 weeks gestation, and 3 weeks lactation)
Frequency of treatment: daily
Duration of test: F1 Generation dosed until Post natal day 85
Doses: 0, 50, 200, and 1000 mg/kg/day
Control Group: yes, concurrent vehicle
NOAEL Maternal Toxicity: = 200 mg/kg bw
NOAEL Teratogenicity: >= 1000 mg/kg bw
Result: The examinations didn't reveal any developmental effect up to the dose level of 1000 mg/kg/day

Method: see below
Year: 2002
GLP: yes
Test substance: Tris-nonylphenyl phosphite

Method: OECD Test Guideline 421 (1998). This study exceeds the OECD 421 study design as follows: (1) enhanced evaluation of toxicity in the F0 generation, including the evaluation of a recovery group of F0 males; (2) evaluation of developmental landmarks in the F1 generation; and (3) following the F1 offspring to adulthood, with continued exposure and assessments of reproductive structures and functions, including potential effects on sperm.

Test procedure: Male and female CD® (Sprague-Dawley) rats (the F0 generation) were administered TNPP orally by gavage at 0, 50, 200, or 1000 mg/kg/day at a dose volume of 5 ml/kg/day in Mazola® corn oil, ten/animals/sex/dose, for two weeks of prebreed exposure (males and females) and two weeks of mating (males and females) for F0 parents. F0 females continued to be dosed for three weeks each of gestation and lactation, as were F1 offspring from weaning through scheduled sacrifice, at approximately 85 days of age. Five additional F0 males per group from the control and 1000 mg/kg/day groups were designated as recovery animals and held without dosing for two weeks, after the F0 male dosing period was completed to evaluate recovery from any possible treatment-related effects identified in the high dose. Body weights and feed consumption for the F0 males and females were recorded at least weekly during the prebreed period for both sexes, for F0 females during gestation and lactation, and F1 offspring from birth through scheduled sacrifice. Clinical signs were recorded at least once daily for all animals. After the two-week prebreed exposure period, animals were randomly mated within treatment groups for a two-week mating period to produce the F1 generation, with continuing exposure. On the day of birth (postnatal day [pnd] 0), anogenital distance (AGD) was measured and body weights recorded for all live F1 pups in all litters. F1 litters were culled on

pnd 4 to yield, as nearly as possible, five males and five females per litter. The culled F1 pups were weighed, euthanized, and necropsied with complete external and visceral examinations. For the remaining F1 pups, survival indices were calculated at least weekly through weaning (pnd 21). At weaning, at least one female and one male (whenever possible) from each F1 litter were randomly selected to continue treatment for approximately seven more weeks, with dosing for F1 selected pups begun on pnd 22 until all pups were at least 85 days of age. F1 postweaning observations and procedures for each retained female included examination for patency of vaginal opening (from pnd 22 until acquisition of vaginal opening). Estrous cyclicity and normality were evaluated by vaginal smears from F1 females taken daily the last three weeks of the postwean exposure period prior to scheduled sacrifice. For each retained F1 male offspring, observations for the cleavage of the balanopreputial gland (preputial separation) began at 35 days of age and continued until acquisition of preputial separation. Andrologic assessments were also performed on the F1 retained males at necropsy. All F0 parental animals, nonselected F1 weanlings, and retained F1 adults were necropsied, with complete histologic evaluation of five selected F0 and F1 males and females in the 0 and 1000 mg/kg/day groups. Histopathology was performed on F1 males and females (five/sex/group) at 0 and 1000 mg/kg/day.

Remark: All F1 pups culled on post natal day 4 were subjected to a complete external and visceral examination, including examination of all thoracic and abdominal organs, bisection of kidneys and heart dissection.

Test substance: Tris-nonylphenyl phosphite
Purity: 99.8%
Supplier : Dover Chemical Corporation

(49)

Species: chicken
Sex: no data
Strain: White Leghorn
Route of administration: Injection of test materials directly into yolk sac of fertile hen's eggs.
Exposure period: One exposure with test substance.
Frequency of treatment: Once.
Duration of test: 5-, 10-, and 18-Day incubation
Doses: 5 mg
Control Group: Yes, with and without vehicle

Method: see below
Year: 1971
GLP: no

Test substance: Tris-nonyl-phenyl phosphite

Method: Technique used described by McLaughlin et al. (1963). Material was dissolved in ethyl ether acetone to which Mazola was added to yield a 10% solution and 0.5 mL of the solution was injected into test eggs. Test groups included untreated eggs, eggs drilled with the needle inserted into the egg sac with nothing injected, eggs injected with sterile water, and eggs injected with corn oil. Two replicate runs were performed.

Remark: Not an adequate developmental toxicity study.
Result: Injection of 5 mg of TNPP per egg into the yolk sac appeared to have little effect on survival of embryos, although the variations between the two replicates were too large to make a firm conclusion. No gross abnormalities were seen in the test groups among chicks that died before hatching or newly hatched chicks.
Reliability: (3) invalid
Not reliable. An unsuitable test system was used.

(54)

5.8.3 Toxicity to Reproduction, Other Studies

Type: One generation study
In Vitro/in vivo: In vivo
Species: rat
Strain: Sprague-Dawley
Sex: male/female
Route of administration: gavage
Exposure period: Males = 4 weeks (2 weeks pre-breeding, 2 weeks mating)
Females = 10 weeks (2 weeks prebeed, 2 weeks mating, 3 weeks gestation, and 3 weeks lactation)
Frequency of treatment: daily
Duration of test: F1 Generation dosed until Post natal day 85
Doses: 0, 50, 200, and 1000 mg/kg/day
Control Group: yes, concurrent vehicle
Result: NOEL Parental: 200 mg/kg/day
NOEL F1 Offspring: 200 mg/kg/day

Method: see below
Year: 2002
GLP: yes
Test substance: Tris-nonylphenyl phosphite

Method: OECD Test Guideline 421 (1998). This study exceeds the OECD 421 study design as follows: (1) enhanced evaluation of toxicity in the F0 generation, including the evaluation of a recovery group of F0 males; (2) evaluation of developmental landmarks in the F1 generation; and (3) following the F1 offspring to adulthood, with continued exposure and assessments of reproductive structures and functions, including potential effects on sperm.

Test procedure: Male and female CD® (Sprague-Dawley) rats (the F0 generation) were administered TNPP orally by gavage at 0, 50, 200, or 1000 mg/kg/day at a dose volume of 5 ml/kg/day in Mazola® corn oil, ten/animals/sex/dose, for two weeks of prebreed exposure (males and females) and two weeks of mating (males and females) for F0 parents. F0 females continued to be dosed for three weeks each of gestation and lactation, as were F1 offspring from weaning through scheduled sacrifice, at approximately 85 days of age. Five additional F0 males per group from the control and 1000 mg/kg/day groups were designated as recovery animals and held without dosing for two weeks, after the F0 male dosing period was completed to evaluate recovery from any possible treatment-related effects identified in the high dose. Body weights and feed consumption for the F0 males and females were recorded at least weekly during the prebreed period for both sexes, for F0 females during

gestation and lactation, and F1 offspring from birth through scheduled sacrifice. Clinical signs were recorded at least once daily for all animals. After the two-week prebreed exposure period, animals were randomly mated within treatment groups for a two-week mating period to produce the F1 generation, with continuing exposure. On the day of birth (postnatal day [pnd] 0), anogenital distance (AGD) was measured and body weights recorded for all live F1 pups in all litters. F1 litters were culled on pnd 4 to yield, as nearly as possible, five males and five females per litter. The culled F1 pups were weighed, euthanized, and necropsied with complete external and visceral examinations. For the remaining F1 pups, survival indices were calculated at least weekly through weaning (pnd 21). At weaning, at least one female and one male (whenever possible) from each F1 litter were randomly selected to continue treatment for approximately seven more weeks, with dosing for F1 selected pups begun on pnd 22 until all pups were at least 85 days of age. F1 postweaning observations and procedures for each retained female included examination for patency of vaginal opening (from pnd 22 until acquisition of vaginal opening). Estrous cyclicity and normality were evaluated by vaginal smears from F1 females taken daily the last three weeks of the postwean exposure period prior to scheduled sacrifice. For each retained F1 male offspring, observations for the cleavage of the balanopreputial gland (preputial separation) began at 35 days of age and continued until acquisition of preputial separation. Andrologic assessments were also performed on the F1 retained males at necropsy. All F0 parental animals, nonselected F1 weanlings, and retained F1 adults were necropsied, with complete histologic evaluation of five selected F0 and F1 males and females in the 0 and 1000 mg/kg/day groups. Histopathology was performed on F1 males and females (five/sex/group) at 0 and 1000 mg/kg/day.

Remark:

All symptoms related to systemic toxicity in F0 and F1 adult rats are detailed in section 5.4 Repeated dose toxicity.

The following is a discussion of F0 parental reproductive toxicity, and F1 offspring toxicity.

F0 Parental Reproductive Toxicity:

Table 5.8.3.1. Summary of F0 Parental Male and Female Reproductive Toxicity

Trisnonylphenyl Phosphite (mg/kg/day)	F0			
	0	50	200	1000
<u>FEMALES</u>				
Precoital interval, days	3.1	2.5	2.3	2.2
Indices:				
Mating	--	--	--	--
Fertility	--	--	--	--
Gestational	--	--	--	--
Gestational length, days	22.2	22.4	22.1	22.3
Death due to dystocia on gd 22	0	0	0	3
No. implant sites/litter	15.80	14.67	16.90	13.50
% postimplantation loss/litter	5.58	15.48	5.68	9.04
No. total pups/litter, pnd 0	15.1	13.8	16.3	12.1
No. live pups/litter, pnd 0	14.9	12.8	15.9	12.0

Trisnonylphenyl Phosphite (mg/kg/day)	F0			
	0	50	200	1000
No. dead pups/litter, pnd 0	0.2	1.0	0.4	0.1
No. females pregnant	10	9	10	10
No. litters on pnd 0	10	8	10	7
No. litters on pnd 21	10	7	10	6
MALES				
Indices: Mating	--	--	--	--
Fertility	--	--	--	--
Gestational	--	--	--	--

-- = no statistically significant difference

Three F0 females at 1000 mg/kg/day were found dead on gd 22, possibly attributable to dystocia. Dystocia was evident due to the inability of the dams to deliver their pups. Examination of the pups at maternal necropsy indicated that they were full term and normal in external appearance. In F0 females, paired ovary weights (absolute and relative to terminal body and brain weights) were significantly decreased at 1000 mg/kg/day but not in F1 females. The increased percent postimplantation loss at 50 mg/kg/day (15.48% versus the control value of 5.58%) was accompanied by a high variance term and is considered due to biologic variation and not treatment (or dose) related.

F1 Offspring Toxicity:

Table 5.8.3.2. Summary of F1 Offspring Toxicity

Trisnonylphenyl Phosphite (mg/kg/day)	F1			
	0	50	200	1000
Stillbirth index	--	--	--	--
Live birth index	--	--	--	--
Survival index (pnd 0-4, precull)	--	--	--	--
Survival index (pnd 4-7, postcull)	--	--	--	--
Survival index (pnd 7-14)	--	--	--	--
Survival index (pnd 14-21)	--	--	--	--
Lactational survival index (pnd 4 postcull-21)	--	--	--	--
No. live pups/litter, pnd 0	14.9	12.8	15.9	21.0
No. live pups/litter, pnd 4 (precull)	14.8	14.3	15.6	12.0*
No. live pups/litter, pnd 7 (postcull)	9.8	10.0	10.0	9.1
No. live pups/litter, pnd 14 (postcull)	9.8	10.0	10.0	9.1
No. live pups/litter, pnd 21 (postcull)	9.8	10.0	10.0	10.0
Sex ratio (% males/litter)	--	--	--	--
AGD/litter, pnd 0:				
Males (mm)	2.04	2.14	2.09	2.10
Females (mm)	0.96	1.00	0.93	0.95
Pup body weight/litter, pnd 0 (g)				
Males	--	--	--	--
Females	--	--	--	--
All pups	--	--	--	--
Pup body weight/litter, pnd 4 (g)				
Males	--	--	--	--
Females	--	--	--	--
All pups	--	--	--	--
Pup body weight/litter, pnd 7 (g)				
Males	--	--	--	--
Females	--	--	--	--
All pups	--	--	--	--
Pup body weight/litter, pnd 14 (g)				
Males	--	--	--	--
Females	--	--	--	--
All pups	--	--	--	--
Pup body weight/litter, pnd 21 (g)				
Males	--	--	--	--

Trisnonylphenyl Phosphite (mg/kg/day)	F1			
	0	50	200	1000
Females	--	--	--	--
All pups	--	--	--	--
Average number of nipples per male pup	0	0	0	0
Average number of areolae per male pup	0	0	0	0
Age at vaginal opening for F1 females (days)	29.4	29.6	30.4	28.7
Age at preputial separation for F1 males (days)	42.4	42.1	42.5	41.0
Estrous cycle length (days)	4.97	4.37	6.23	4.78
Histopathology:				
Reproductive Organs:	Males ^a	--	--	--
	Females ^b	--	--	--
Systemic Organs:	Males ^a	--	--	↑ ^b
	Females ^b	--	--	--
Andrology				
% Motile sperm	--	--	--	--
% Agressively motile sperm	--	--	--	--
Epididymal sperm concentration ^a	--	--	--	--
Testicular spermatid head count ^a	--	--	--	--
Daily sperm production ^a	--	--	--	--
Efficiency of daily sperm production ^a	--	--	--	--

^a Indicated parameters were assessed in control and high dose animals only

↑^b Presence of renal corticomedullary mineralization in two F1 males at 1000 mg/kg/day versus 0 at 0 mg/kg/day. F1 females exhibited the same findings with the same incidence and severity at both 0 and 1000 mg/kg/day.

--- = No statistically significant difference

* = Statistically significantly different from control group value at p<0.05

There was evidence of F1 offspring toxicity at 1000 mg/kg/day, expressed as reduced live litter size on pnd 4. The result at pnd 0 at 1000 mg/kg/day was not statistically significantly different from the control group, however, the values were the same as at pnd4. There were no other indications of F1 offspring toxicity either pre- or postnatally through lactation, with no effects on offspring survival, AGD, sex ratio (% males) per litter, average number of nipples/areolae for male pups, or body weights per litter. Age at acquisition of F1 male preputial separation and F1 female vaginal opening was equivalent across all groups. There were no effects on body weights for the F1 males and females at sacrifice on pnd 21 at any dose.

Result:

Three of ten pregnant F0 parental females from the 1000 mg/kg/day group were found dead on Day 22 of gestation. These deaths may have been related to dystocia, since the dams appeared to be unable to deliver their normal appearing pups. In addition, absolute ovarian weights and ovarian weights relative to terminal body or brain weights were reduced in F0 females at 1000 mg/kg/day. No other consistent, treatment-related effects were observed. There was no evidence of F0 reproductive toxicity in males. The only evidences of F1 offspring toxicity pre- or postnatally were observed at 1000 mg/kg and were a reduced litter size on pnd 4 (also observed on pnd 0 but not of statistical significance), renal histopathology in F1 adult males and a significantly decreased paired epididymides weight,

relative to terminal body weights in F1 male rats. This last finding is of uncertain toxicological significance, since there were no changes in epididymal weight in the F0 generation or in absolute organ weight in the F1 generation. No effects were seen on the developmental landmarks, including the time to vaginal opening or preputial separation, or estrous cycle normality or length. Therefore, under the conditions of this study in rats, the no observable adverse effect levels (NOAELs) for systemic parental toxicity and for reproductive and offspring toxicity were 200 mg/kg/day.

Test substance: Tris-nonylphenyl phosphite
Purity: 99.8%
Supplier : Dover Chemical Corporation

Reliability: (1) valid without restriction

(49)

Type: Two generation study
In Vitro/in vivo: In vivo
Species: rat
Strain: no data
Sex: male/female
Route of administration: oral feed
Exposure period: 2 years
Frequency of treatment: daily
Duration of test: 2 years
Doses: Designed to be equivalent to approximately 1000, 3300, and 10,000 ppm in the diet when the rats reached maturity (0, 50, 167, and 500 mg/kg/day)

Control Group: yes, concurrent no treatment

Result: NOAEL Parental: 10000 ppm (500 mg/kg)
NOAEL F1 Offspring: 10000 ppm (500 mg/kg)
NOAEL F2 Offspring: 10000 ppm (500 mg/kg)

Method: see below
Year: 1961
GLP: no
Test substance: Tri (polynonylphenyl) phosphite

Method: Rats: 200 weanling rats were distributed into four groups of 25 males and 25 females each. Appearance, behavior, and survival noted daily. Records of body weight were made weekly for first 12 weeks, and 4 week intervals thereafter. At 12 weeks and at approximately half-yearly intervals clinical examinations were made in 10 rats of each sex in the control and highest test level groups and 5 of each sex in the two lower levels. Clinical tests included erythrocyte and leukocyte counts; blood hemoglobin, hematocrit, sugar, and nonprotein nitrogen determinations; and urine protein, sugar and sediment. Blood cholesterol levels and prothrombin time were made at several intervals. Reproductive studies were initiated after 100 days, and 10 representative rats from each sex were placed on the same testing as the parents. Their young, and in turn the descendant of that generation, were carried through similar experiments, making a total of three descendant generations studied. Reproduction and lactation experiments on the F0 generation were carried through six matings. The F1 and F2 generations were carried through only two matings and the F3 generation was not mated. Records were kept of the date

of birth, the number of pups born, their weight and survival during lactation. The criteria employed for evaluating the performance of the rats were a series of indexes for fertility, gestation, viability and lactation. Reproductive organs (testes and ovaries) were autopsied and weighted.

- Remark:** Same study as described in Repeated Dose Toxicity (Section 16b).
- Result:** At the highest concentration, body weight gain was slightly reduced at 12 weeks for the F0, F2, and F3 males and for the F3 females. No significant treatment-related changes were observed for mortality, food consumption, hematology, clinical chemistry, or anti-cholinesterase activity. There were no indication of any changes in fertility, gestation, viability, or lactation for the F0 animals and the descendent generations, except for a possible diminution in the number of pups born per litter in the F1 and F2 high dose groups, and a small decrease in the fertility and viability indexes at this same high dose level exposure ; however, no statistical test was used. There was no significant change from control animals for any generation with respect to organ weights and dose-related findings at necropsy.
- Test substance:** Tri (polynonylphenyl) phosphite.
Purity not specified
Supplier : the Naugatuck Chemical Division of the United States Rubber Company
- Reliability:** (2) valid with restrictions
Non-GLP and did not follow specific OECD test guidelines. However, it was an acceptable, well-documented study report which meets basic scientific principles.

(52)

5.10 Exposure Experience

- Remark:** No specific hazard known to human exposed to the substance during preparation.
- Source:** GREAT LAKES CHEMICAL ITALIA MILAN
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

9.0 References

- (1) Ciba Geigy (1992) Report on the acute toxicity test of Irgafos TNPP on Daphnia (Daphnia magna Straus 1820). Unpublished report 928135 (28/8/92)
- (2) Ciba Geigy (1992) Report on the acute toxicity test of IRGAFOS TNPP to Zebra-Fish (Brachydanio rerio). Unpublished report 928134 (28/8/92)
- (3) Ciba MSDS
- (4) Ciba MSDS.
- (5) CIBA-Geigy (1988). Report on the test for acute toxicity test of TK 10417 to Golden Orfe (Leuciscus idus), unpublished report, Report No 88 41 26. Basel, Switzerland, June 20, 1999, CIBA-GEIGY: 9.
- (6) CIBA-Geigy (1988). Report on the test for inhibitory concentration of TK 10417 [TNPP] on aerobic bacteria (OECD-GUIDELINE N° 209),

- unpublished report, Project No 88 41 73. Basle, Switzerland, June 08, 1988, CIBA: 8.
- (7) CIBA-Geigy (1992a). Report on the growth inhibition test of IRGAFOS TNPP to green algae (*Scenedesmus subspicatus*), unpublished report, Report No 92 81 36. Basel, Switzerland, August 28, 1992, CIBA-GEIGY: 12.
 - (8) CIBA-Geigy (1994). Report on the test for ready biodegradability of IRGASTAB CH 55 in the carbondioxide evolution test, unpublished report, Test No 94 80 08. Basle, Switzerland, April 18, 1994, CIBA-GEIGY: 11.
 - (9) CLOGP, Daylight Chemical Information Systems, Inc.
 - (10) Crompton, WESTON TNPP Phosphite, product information, revised 08-01-2003
 - (11) Deparade (1990), Salmonella/mammalian-microsome mutagenicity test (OECD conform), Test number : 89547. Ciba-Geigy Limited
 - (12) Geleick (1990), Gene mutation test with Chinese hamster cells V79, Test number 894549. Ciba-Geigy Limited.
 - (13) Gudi, R. (2001). Unpublished report no AA37MU.331.BTL entitled "In vitro mammalian chromosome aberration test" dated August 22, 2001 for General Electric Specialty Chemicals, Morgantown, WV from BioReliance Corp., Rockville, MD.
 - (14) Guterson, D.S. (2001). Unpublished report no. 20000865/20002536 entitled "Algal growth inhibition test (OECD 201) on trisnonylphenyl phosphite (TNPP)", dated August 27, 2001 for General Electric Company, Pittsfield, MA from HydroQual Laboratories, Ltd., Calgary, Alberta, Canada
 - (15) Guterson, D.S. (2001). Unpublished report no. 20000865/20002537 entitled "Acute toxicity of trisnonylphenyl phosphite (TNPP) to *Daphnia magna*", dated August 27, 2001 for General Electric Company, Pittsfield, MA from HydroQual Laboratories, Ltd., Calgary, Alberta, Canada
 - (16) Guterson, D.S. (2001). Unpublished report no. 20000865/20002538 entitled "Acute toxicity of trisnonylphenyl phosphite (TNPP) to fish (Rainbow trout; OECD203)", dated August 27, 2001 for General Electric Company, Pittsfield, MA from HydroQual Laboratories, Ltd., Calgary, Alberta, Canada
 - (17) Guterson, D.S. (2001). Unpublished report no. 20000865/20002539a entitled "Ready biodegradability of trisnonylphenyl phosphite (TNPP: OECD301D; CAS# 26523-78-4)", dated November 17, 2001 for General Electric Company, Pittsfield, MA from HydroQual Laboratories, Ltd., Calgary, Alberta, Canada
 - (18) Hachiya, N. 1987. Evaluation of chemical genotoxicity by a series of short-term tests. *Akita J. Med.* 14(2): 269-292.
 - (19) Hagemann (1992), Skin sensitisation test in the Guinea pig, Test n° 924058. Ciba-Geigy Limited.
 - (20) Hartmann (1992), Acute dermal toxicity in the rat, Test n° 924059, TK 10417 (Irgafos TNPP). Ciba-Geigy Limited.
 - (21) Internal Reference GREAT LAKES CHEMICAL ITALIA MILAN

- (22) Internal Reference.
- (23) Internal reference. GREAT LAKES CHEMICAL ITALIA MILAN
- (24) Kobel (1983), Acute intraperitoneal LD50 in rat, Test n° 831156, TK 10417 (Irgafos TNPP). Ciba-Geigy Limited.
- (25) Majlathova, L. 1981. (Lekarskej Fak., Univ.Komenskeho, 03753 Martine, Czech.). Evaluation of alkyl phenylphosphite antioxidants by an acute peroral experiment on mice and rats and by epicutaneous and conjunctival test on rabbits. Bratisl. Lek. Listy. 76(3): 315-326.
- (26) McLaughlin et al., Tox. Appl. Pharm. 5: 760 (1963)
- (27) MDL information systems.
- (28) Miller LC, Tainter ML, Proc. Soc. Exptl. Biol. And Med., 57, 261 (1944)
- (29) MSDS Ciba.
- (30) MSDS Rhone Poulenc
- (31) MSDS Uniroyal
- (32) Phoenix Chemical Laboratory, Inc. (1997). Report No. 7 4 24 8, Page 8 of 22.
- (33) Pittsburg Testing Laboratory (1978) Report No 787742, July 12, 1978
- (34) Pritt, S. (2001). Unpublished report n°01-4176-G4 entitled "Tris-nonylphenol (TNPP) : Buehler sensitisation test - (OECD 406)", dated October 3, 2001 for General Electric Company from Toxikon Corporation, Bedford, MA.
- (35) Reimer, G.J. (2001). Unpublished report 171 1234BP (BC Research Inc. no. 406 0001) entitled "Physical/chemical property of tris-nonylphenyl phosphite (TNPP) [26523-78-4]: Boiling temperature", dated August 10, 2001 for General Electric Company, Pittsfield, MA from BC Research Inc., Vancouver, BC, Canada
- (36) Reimer, G.J. (2001). Unpublished report no. 171 1234 entitled "Physical/chemical property of tris-nonylphenyl phosphite (TNPP) [26523-78-4]: n-Octanol/water partition coefficient - Expert statement", dated August 10, 2001 for General Electric Company, Pittsfield, MA from Reimer Analytical and Associates, Inc. Vancouver, BC, Canada
- (37) Reimer, G.J. (2001). Unpublished report no. 171 1234 entitled "Physical/chemical property of tris-nonylphenyl phosphite (TNPP) [26523-78-4]: Photolytic stability in water - Test waiver statement", dated September 26, 2001 for General Electric Company from Reimer Analytical and Associates Inc., Vancouver, BC, Canada
- (38) Reimer, G.J. (2001). Unpublished report no. 171 1234Hy (BC Research Inc. no. 406 0001) entitled "Physical/chemical property of tris-nonylphenyl phosphite (TNPP) [26523-78-4]: Hydrolysis as a function of pH", dated August 10, 2001 for General Electric Company, Pittsfield, MA from BC Research Inc., Inc. Vancouver, BC, Canada
- (39) Reimer, G.J. (2001). Unpublished report no. 171 1234MP (BC Research

Inc. no 406 0001) entitled "Physical/chemical property of tris-nonylphenyl phosphite (TNPP) [26523-78-4]: Melting (pour) point", dated August 10, 2001 for General Electric Company, Pittsfield, MA, from BC Research Inc., Vancouver, BC, Canada.

- (40) Reimer, G.J. (2001). Unpublished report no. 171 1234SW (BC Research Inc. no. 406 0001) entitled "Physical/chemical property of tris-nonylphenyl phosphite (TNPP) [26523-78-4]: Solubility in water", dated August 10, 2001 for General Electric Company, Pittsfield, MA from BC Research Inc., Inc. Vancouver, BC, Canada
- (41) San, R.H.C. and Clarke, J.J (2001). Unpublished report no AA37MU.702.BTL entitled "In vitro mammalian cell gene mutation test (L5178Y/TK+/- mouse lymphoma assay)" dated August 21, 2001 for General Electric Specialty Materials, Morgantown, WV from BioReliance Corp., Rockville, MD
- (42) Seifert (1982), Eye irritation in thr rabbit after a single application of TK 10 417, Ciba-Geigy Limited.
- (43) Seifert (1982), Skin irritation in the rabbit after a single application of TK 10 417. Ciba-Geigy Limited.
- (44) Staples, C.A. 2001. Unpublished report entitled "Fugacity Modeling to Estimate Transport Between Environmental Compartments for Tris-nonylphenyl phosphite (TNPP) (CAS Reg. No. 26523-78-4)," dated November 10, 2001, for General Electric Company, Pittsfield, MA from Assessment Technologies, Inc. Fairfax, VA.

Report cites the following additional references:

Mackay, D. et al. 1996a. Assessing the fate of new and existing chemicals: a five-stage process. Environ. Toxicol. Chem. 15(9): 1618-1626.

Mackay, D. et al. 1996b. Evaluating the environmental fate of a variety of types of chemicals using the EQC model. Environ. Toxicol. Chem. 15(9): 1627-1637.

Meylan, W. and PH Howard. 1999a. User's Guide for MPBPVP, Version 1.4. Syracuse Research Corporation. North Syracuse, New York. December, 1999.

Meylan, W. and PH Howard. 1999b. User's Guide for KOWWIN, Version 1.6. Syracuse Research Corporation. North Syracuse, New York. July, 1999.

Meylan, W. and PH Howard. 1999c. User's Guide for WSKOWWIN, Version 1.3. Syracuse Research Corporation. North Syracuse, New York. April, 1999.

Meylan, W. and PH Howard. 2000a. User's Guide for AOPWIN, Version 1.9. Syracuse Research Corporation. North Syracuse, New York. March, 2000.

Meylan, W. and PH Howard. 2000b. User's Guide for BIOWIN, Version 4.0. Syracuse Research Corporation. North Syracuse, New York. February, 2000.

Reimer Analytical & Associates, Inc. 2001a. Physical-Chemical Property of TNPP (26523-78-4). Hydrolysis as a Function of pH (OECD 111). Study

No. 171-1234HY, Vancouver, BC, Canada, August 10, 2001.

Reimer Analytical & Associates, Inc. 2001b. Physical-Chemical Property of TNPP (26523-78-4). Melting Point (OECD 102). Study No. 171-1234MP, Vancouver, BC, Canada, August 10, 2001.

Reimer Analytical & Associates, Inc. 2001c. Physical-Chemical Property of TNPP (26523-78-4). Solubility in Water (OECD 105). Study No. 171-1234SW, Vancouver, BC, Canada, August 10, 2001.

Reimer Analytical & Associates, Inc. 2001d. Physical-Chemical Property of TNPP (26523-78-4). n-Octanol/Water Partition Coefficient (Expert Statement) Study No. 171-1234, Vancouver, BC, Canada, August 10, 2001.

Syracuse Research Corporation. 2000. User's Guide for Estimation Programs Interface for Windows, Version 3, Syracuse Research Corporation. North Syracuse, New York.

- (45) Strasser (1990), Chromosome studies on Chinese hamster ovary cell line CCL61 in vitro, Test n° 894548
- (46) Tay, C.H. (2001a). Unpublished report no. 01-4176-G1 entitled "Acute dermal toxicity study", dated December 4, 2001 for General Electric Company from Toxikon Corporation, Bedford, MA.
- (47) Tay, C.H. (2001b). Unpublished report n°01-4176-G2 entitled "Tris-nonylphenol (TNPP) : Acute dermal irritation/corrosion - (OECD 404)", dated October 10, 2001 for General Electric Company from Toxikon Corporation, Bedford, MA.
- (48) Tay, C.H. (2001c). Unpublished report n°01-4176-G3 entitled "Tris-nonylphenol (TNPP) : Acute eye irritation/corrosion - (OECD 405)", dated October 30, 2001 for General Electric Company from Toxikon Corporation, Bedford, MA.
- (49) Tyl, R.W., Hamby, B.T., Myers, C.B., Marr, M.C. (2002). Unpublished report no 65C-07895.300 entitled "Reproductive/Developmental Toxicity Screening Test of Trisnonylphenyl Phosphite (TNPP) Administered via Oral Gavage to CD® (Sprague-Dawley) Rats (Modified OECD 421)" dated April 10, 2002 for General Electric Company, Pittsfield, MA from RTI, Research Triangle Park, NC.
- (50) United States Testing Company (1990) report No 034146, February 6, 1990
- (51) Unpublished report (1957) entitled Toxicological studies with TNPP: (d) Acute Oral LD50; (e) Subacute Feeding Tests, conducted by Food and Drug Research Laboratories for Naugatuck Chemical Corporation (Division of U.S. Rubber Company).
- (52) Unpublished report (1961) entitled Two-year feeding studies on TNPP in rats and dogs conducted by Food and Drug Research Laboratories for Naugatuck Chemical Corporation (Division of U.S. Rubber Company).
- (53) Unpublished report (1965) entitled Acute Oral Administration of Nonylated Phenyl Phosphite (Samples #1 and #2), Mark 488, and Mark 492 to Rats, conducted by Hill Top Research, Inc. Argus Chemical dated June 7, 1965.
- (54) Unpublished report (1971) entitled The Effects of Three Samples on the Survival Rate Chick Embryos conducted by Food and Drug Research Laboratories for Weston Chemicals, Inc.

- (55) Wagner, V.O. (2001). Unpublished report no AA37MU.502.BTL entitled "Bacterial reverse mutation assay" dated August 22, 2001 for General Electric Specialty Materials, Morgantown, WV from BioReliance Corp., Rockville, MD.