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CHEMICAL CHALLENGE PROGRAM

ROBUST SUMMARY

1,3-Isobenzofurandione, 5,5'-[(1-methylethylidene)
bis(4,1-phenyleneoxy)]bis-

(Bisphenol A Dianhydride; CAS RN 38103-06-9)

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Prepared for:

U.S. Environmental Protection Agency

Washington, D.C., USA

December 29, 2005

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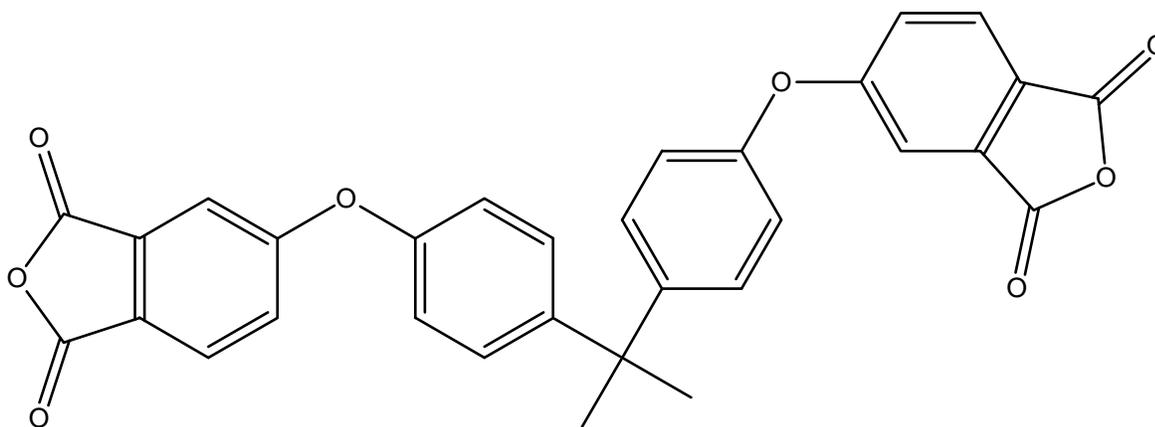
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CHEMICAL IDENTITY AND USE INFORMATION**CAS RN:**

38103-06-9

CHEMICAL NAME

1,3-Isobenzofurandione, 5,5'-[(1-methylethylidene)bis(4,1-phenyleneoxy)]bis-
(hereafter called Bisphenol A Dianhydride)

STRUCTURE, MOLECULAR FORMULA, MOLECULAR WEIGHTMolecular Formula: $C_{31}H_{20}O_8$

Molecular Wt.: 520.49

OTHER CHEMICAL IDENTITY INFORMATION

Bisphenol A Dianhydride

4,4'-((Isopropylidene)bis(p-phenyleneoxy))diphthalic dianhydride

2,2-bis(4-(3,4-dicarboxyphenoxy)phenyl)propanedianhydride

BPA-DA

Bisphenol-A diphthalic anhydride

Biphenol Dianhydride

PURITY

Typical purity of 4,4'-BPA-DA is 97 wt%. Mono N-methylimide of BPA-DA comprise approximately 2 wt% yielding a purity of all isomers > 98 wt%.

USE PATTERN

Bisphenol A Dianhydride (BPA-DA) is a chemical intermediate that is primarily used as a reactive intermediate to make high molecular weight polyetherimide polymers.

FINAL TEST STATUS

Bisphenol A Dianhydride		Information	OECD Study	GLP	Other Study	Estimation Method	Acceptable	SIDS Testing Required
CAS RN: 38103-06-9								
STUDY		Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N
PHYSICAL AND CHEMICAL DATA								
1.0	Melting Point	Y	Y	Y	N	N	Y	N
2.0	Boiling Point	Y	Y	Y	N	N	Y	N
3.0	Vapor Pressure	Y	N	N	Y	Y	Y	N
4.0	Partition Coefficient	Y	N	N	Y	Y	Y	N
5.0	Water Solubility	Y	N	N	Y	Y	Y	N
ENVIRONMENTAL FATE AND PATHWAY								
6.0	Photodegradation	Y	N	N	Y	Y	Y	N
7.0	Stability in Water	Y	Y	N	N	N	Y	N
8.0	Transport and Distribution	Y	N	N	Y	Y	Y	N
9.0	Biodegradation	Y	Y	Y	N	N	Y	N
ECOTOXICITY								
10.0	Acute Toxicity to Fish	Y	Y	Y	N	N	Y	N
11.0	Toxicity to Algae	Y	Y	Y	N	N	Y	N
12.0	Acute Toxicity to Daphnia	Y	Y	Y	N	N	Y	N
TOXICITY								
13.0	Acute Toxicity	Y	N	N	Y	N	Y	N
14.0	Genotoxicity <i>In Vitro</i> or <i>In Vivo</i> (Chromosome Aberration Test)	Y	Y	Y	N	N	Y	N
15.1	Genotoxicity <i>In Vitro</i> (Bacterial Test)	Y	N	Y	Y	N	Y	N
15.2	Genotoxicity <i>In Vitro</i> (Mammalian Cells)	Y	Y	Y	N	N	Y	N
16.0	Repeated Dose Toxicity	Y	N	Y	Y	N	Y	N
17.0	Reproductive Toxicity	Y	Y	Y	N	N	Y	N
18.0	Developmental Toxicity / Teratogenicity	Y	N	Y	Y	N	Y	N

ROBUST SUMMARY**PHYSICAL AND CHEMICAL DATA****1.0 MELTING POINT**

Value:	185-190 °C
Decomposition:	Yes [] No [X] Ambiguous []
Sublimation:	Yes [] No [X] Ambiguous []
Method:	OECD Test Guideline 102 (1993)
GLP:	Yes [X] No [] ? []
Test Substance:	4,4'- Bisphenol A dianhydride (Bisphenol A Dianhydride; BPA-DA; CAS RN 38103-06-9); Lot UI0054 from General Electric Plastics; Purity: 97%.
Remarks:	<p>The melting point (MP) of a calibration substance (phenanthrene) and BPA-DA was determined in duplicate as follows: The sample was placed at the sealed end of a glass capillary tube to a height of approximately 2 mm. The tube was placed in the melting point apparatus, the heating block of which was heated to approximately 5 °C below the MP (estimated in a preliminary study). The contents of the capillary were heated at 1 °C/min and the temperatures of Stages A (wet point), B (shrinkage point), C (collapse point), D (liquefying point), and E (final stage) were recorded. It was also noted if the sample changed color or appeared to decompose during the test. The temperature range from Stage A-E is the melting point.</p> <p><u>Results:</u> The calibration substance, phenanthrene, gave an average melting point of 97 - 99 °C, which, when compared to the expected MP of 99 - 101 °C, indicated a bias of 2 °C. The MP results for BPA-DA were elevated 2°C to account for this bias. The observed bias adjusted average MP of BPA-DA was 185 - 190 °C. No color change was observed.</p>
Reference:	Reimer, G.J. (2004). Unpublished Report No. RAA7213 1449-MP (Vizon Project No. SP7213 1449-MP) entitled "Physical/chemical property of BPA-DA, CAS # 38103-06-9: Melting Point (OECD 102)", dated April 22, 2004 for General Electric Company, Pittsfield, MA, USA; from Vizon SciTec Inc., Vancouver, BC, Canada.
Reliability:	(Klimisch Code 1) Valid without restrictions.

2.0 BOILING POINT

Value:	>314°C
Pressure:	101.3 kPa
Decomposition:	Yes [] No [X] Ambiguous []
Method:	OECD Test Guideline 103 (1993)
GLP:	Yes [X] No [] ? []
Test Substance:	4,4'-Bisphenol A dianhydride (Bisphenol A Dianhydride; BPA-DA; CAS RN 38103-06-9); Lot UI0054 from General Electric Plastics; Purity: 97%.

Remarks:	Duplicate samples of BPA-DA and ethylene carbonate, a reference substance, were placed in Pyrex test tubes along with a few boiling chips. A thermocouple tip was placed in the sample at the bottom of each tube and connected to the digital temperature logger. The tube and thermocouple were placed in aluminium block on an asbestos screen. A gas burner was used to heat the block and the fume hood door was closed. General observations were recorded, such as change in color, presence of smoke and if the substance boiled. The burner was turned off when the substance boiled or the thermocouple reading was 310 °C. This method was calibrated by using ethylene carbonate (EC) as a reference substance. The observed duplicate boiling points of the EC were 245°C and 244°C for an average of $245 \pm 1^\circ\text{C}$ at 101.3 kPa. This compared to the reported BP of 248°C at 101.3 kPa, indicating an average deviation/bias of 3°C. The duplicate BPA-DA warming curves did not show a distinct boiling plateau up to the maximum temperature of 311°C. This was supported by the fact that BPA-DA was not observed to boil during the test. No BPA-DA color change was observed during the test. Therefore, the bias-corrected BP of BPA-DA was greater than 314°C at 101.3 kPa. BP measurement at reduced pressure was not performed because the calculated BP at atmospheric pressure was greater than 300°C, which is the regulatory limit set by the US EPA (EPA, 1999) and Environment Canadian (CEPA, 1993).
Reference:	Reimer, G.J. (2004). Unpublished Report No. RAA7213 1449-BP (Project No. SP7213 1449-BP) entitled “Physical/chemical property of BPA-DA, CAS # 38103-06-9: Boiling Point (OECD 103)”, dated April 22, 2004 for General Electric Company, Pittsfield, MA, USA; from Vizon SciTec Inc., Vancouver, BC, Canada.
Reliability:	(Klimisch Code 1) Valid without restrictions.

3.0 VAPOR PRESSURE

Value:	5E-18 Pa at 25°C
Decomposition:	Yes [] No [] Ambiguous [] Not Applicable [X]
Method:	Calculated [X] Measured []
GLP:	Yes [] No [X] ? []
Test Substance:	4,4'-Bisphenol A dianhydride (Bisphenol A Dianhydride; BPA-DA; CAS RN 38103-06-9).
Remarks:	Vapor pressure was calculated by computer software from Advanced Chemistry Development Inc., as the calculated VP of BPA-DA (5E-18 Pa at 25°C) was less than the detection limit ($\sim 1\text{E-}5$ Pa) of the most sensitive vapor pressure method (gas saturation method; OECD 104, 1995). The model is based on the boiling point at 760 mmHg. Boiling points determined at other pressures are recalculated using the Hass and Newton formula. The boiling point for BPA-DA was previously determined to be $>314^\circ\text{C}$ at 101.3 kPa (760 mmHg) (Reimer, 2004b). The following equation is used to calculate the vapor pressure (Δ_{vap}):

$$\Delta_{vap}H(T_b) = [(\varphi * 2.303 * R * T_b(K))] * 0.839594 + 3.9039.$$

ACD values show a higher correlation coefficient, when plotted against experimental values, compared to using the Watson method. Also, the ACD values show generally lower deviations from the experimental vapor pressures compared to the ‘Watson values.’ The OECD Guideline 104 supports the use of the Watson correlation for the calculation of VP, but does not specifically reject other calculation methods.

Using the ACD method, the vapor pressure for BPA-DA was determined to be 5E-18 Pa at 25°C.

Reference: Reimer, G.J. (2004). Unpublished Report No. RAA7213 1449-VP (Project No. SP7213 1449-VP) entitled “Physical/chemical property of BPA-DA, CAS # 38103-06-9: Vapor Pressure(OECD 104)”, dated March 2, 2004 for General Electric Company, Pittsfield, MA, USA; from Reimer Analytical and Associates Inc., Vancouver, BC, Canada.

Reimer, G.J. (2004b). Unpublished Report No. RAA7213 1449-BP (Project No. SP7213 1449-BP) entitled “Physical/chemical property of BPA-DA, CAS # 38103-06-9: Boiling Point (OECD 103)”, dated April 22, 2004 for General Electric Company, Pittsfield, MA, USA; from Vizion SciTec Inc., Vancouver, BC, Canada..

Reliability: (Klimisch Code 2) Valid with restrictions, calculated value.

4.0 PARTITION COEFFICIENT ($\text{Log}_{10}P_{ow}$)

Log Pow: Not applicable

Temperature: Not applicable

Method: Expert Statement based on OECD Test Guideline 107 (1995)

GLP: Yes [] No [] ? [] Not applicable [X]

Test Substance: 4,4'-Bisphenol A dianhydride (Bisphenol A Dianhydride; BPA-DA; CAS RN 38103-06-9).

Remarks: The hydrolytic stability of the test substance BPA-DA was investigated in a previous study (Reimer, 2005b) by preparing solutions of BPA-DA in acetonitrile (CH₃CN) with aqueous components that were deionized water (DIW; pH 5 – 6), DIW adjusted to pH 2, or DIW adjusted to pH 9. The concentrations of the aqueous components were varied from 1 to 40% v/v. Analysis of these solutions by HPLC-UV showed that the half-lives of BPA-DA were less than 14 h, 11 h, and 7 h at 23°C under these neutral (pH 5 – 6), acidic, and basic conditions, respectively. Therefore, this hydrolytic instability of BPA-DA precluded the performance of the OECD Guideline 107 *n*-octanol/water partition coefficient test (OECD, 1995).

Reference: Reimer, G.J. (2005). Unpublished Report No. RAA7213 1449-Pow entitled “Physical/chemical property of BPA-DA [CAS # 38103-06-9]: *n*-Octanol/Water Partition Coefficient (OECD 107)”, dated

March 30, 2005 for General Electric Company, Pittsfield, MA, USA; from Reimer Analytical and Associates Inc., Vancouver, BC, Canada.

Reimer, G.J. (2005b). Unpublished Report No. RAA7213 1449-Hy entitled “Physical/chemical property of BPA-DA [CAS # 38103-06-9]: Hydrolytic Stability (OECD 111)”, dated June 10, 2005 for General Electric Company, Pittsfield, MA, USA; from Vizon SciTec Inc., Vancouver, BC, Canada.

Reliability: (Klimisch Code 2) Valid with restrictions; Expert statement; value could not be determined.

5.0 WATER SOLUBILITY

5.1 SOLUBILITY

Value: Not applicable

Temperature: Not applicable

Description: Miscible []; Of very high solubility []; Of high solubility []; Soluble []; Slightly soluble []; Of low solubility []; Of very low solubility []; Not soluble []

Method: Expert Statement based on OECD Test Guideline 105

GLP: Yes [] No [] ? [] Not applicable [X]

Test Substance: 4,4'-Bisphenol A dianhydride (Bisphenol A Dianhydride; BPA-DA; CAS RN 38103-06-9)

Remarks: The hydrolytic stability of the test substance BPA-DA was investigated in a previous study (Reimer, 2005b) by preparing solutions of BPA-DA in acetonitrile (CH₃CN) with aqueous components that were deionized water (DIW; pH 5 – 6), DIW adjusted to pH 2, or DIW adjusted to pH 9. The concentrations of the aqueous components were varied from 1 to 40 vol. %. Analysis of these solutions by HPLC-UV showed that the half-lives of BPA-DA were less than 14 h, 11 h, and 7 h at 23°C under these neutral (pH 5 – 6), acidic, and basic conditions, respectively (Reimer, 2005b). Therefore, this hydrolytic instability of BPA-DA precluded the performance of the OECD Guideline 105 water solubility test (OECD, 1995).

Reference: Reimer, G.J. (2005). Unpublished Report No. RAA7213 1449-WS entitled “Physical/chemical property of BPA-DA [CAS # 38103-06-9]: Water Solubility (OECD 105)”, dated March 29, 2005 for General Electric Company, Pittsfield, MA, USA; from Reimer Analytica & Associate Inc., Vancouver, BC, Canada.

Reimer, G.J. (2005b). Unpublished Report No. RAA7213 1449-Hy entitled “Physical/chemical property of BPA-DA [CAS # 38103-06-9]: Hydrolytic Stability (OECD 111)”, dated June 10, 2005 for General Electric Company, Pittsfield, MA, USA; from Vizon SciTec Inc., Vancouver, BC, Canada.

Reliability: (Klimisch Code 2) Valid with restrictions; Expert statement; value could not be determined

5.2. pH VALUE, pKa VALUE

No studies were found.

ENVIRONMENTAL FATE AND PATHWAYS

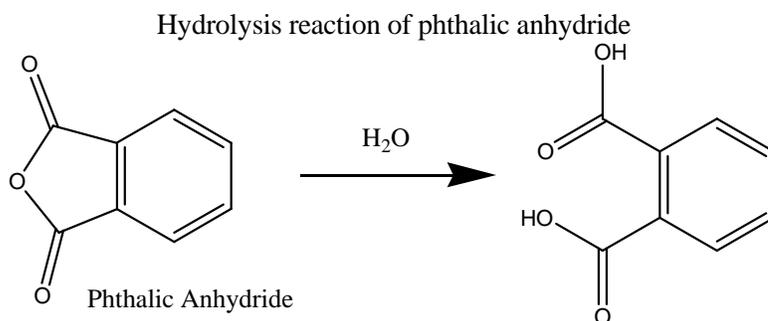
6.0 PHOTODEGRADATION

Method: Calculated [X] Measured []
 GLP: Yes [] No [X] ? []
 Test Substance: 1,3-Isobenzofurandione, 5,5'- (1-methylethylidene)bis(4,1-phenyleneoxy) bis-[Bisphenol A Dianhydride; BPA-DA; CAS RN 38103-06-9]
 Concentration: Not applicable
 Temperature °C: Not applicable
 Direct photolysis: Not applicable
 Indirect photolysis: Not applicable
 Breakdown products: Not applicable
 Value: Overall OH Rate Constant (k_{phot}) = 15.9214 E-12 cm³/molecule-sec
 Half-life ($t_{1/2}$) = 8.062 Hrs (12-hour day; 1.5E+06 OH/cm³)
 Remarks: Atmospheric photo-oxidation potential, mediated by reaction with hydroxyl radicals, was estimated using the submodel, Atmospheric Oxidation Potential for Windows (AOPWIN v.1.91, EPIWIN v3.11, USEPA, 2003). The SAR methods rely on structural features of the subject molecule. The model calculates a second-order half-life with units of cm³/molecules-sec. A pseudo-first order photo-degradation rate is in turn based on the second order rate of reaction (cm³/molecules-sec) with hydroxyl radicals (HO•), assuming first-order kinetics and an HO• concentration of 1.5 E+06 molecules/cm³ and 12 hours of daylight. Pseudo-first order half-lives ($t_{1/2}$) were then calculated as follows:

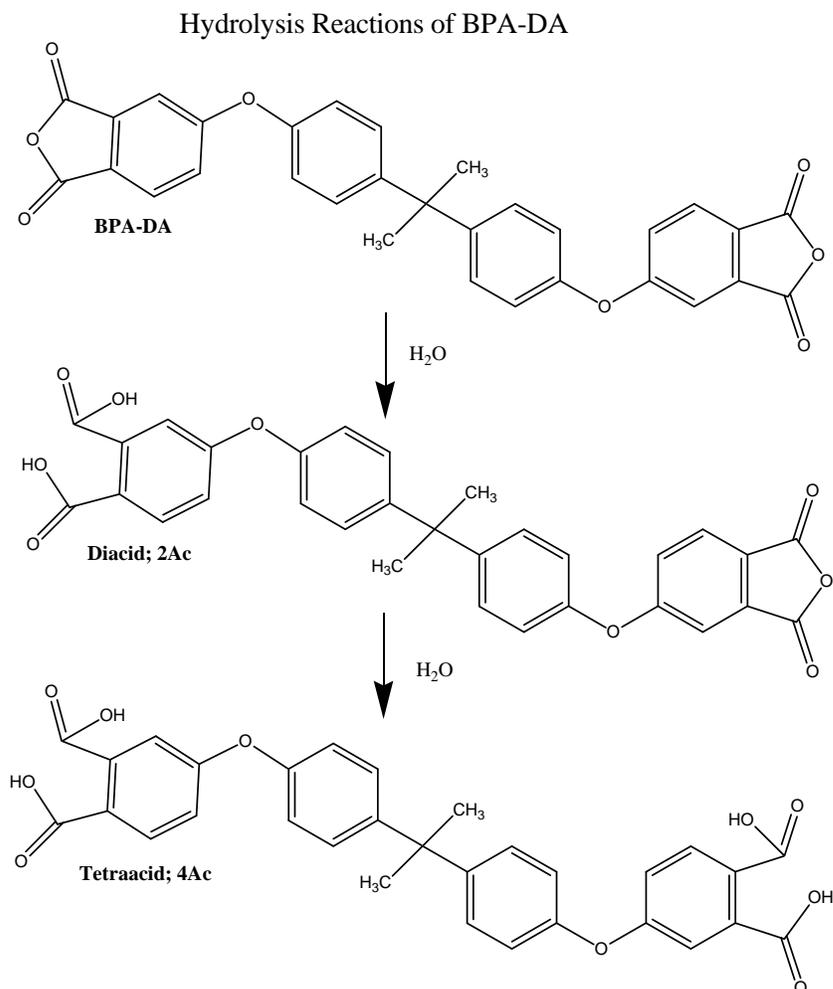
$$t_{1/2} = 0.693 / [k_{\text{phot}} \times \text{HO} \cdot \times 12\text{-hr} / 24\text{-hr}].$$
 The EPIWIN model (v 3.11) was run using the following measured physical chemical properties:
 Vapor pressure = 4E-020 mm Hg;
 Boiling point = 314°C; and
 Melting point 187.5°C.
 Reference: U.S. EPA (U.S. Environmental Protection Agency). 2003. EPI Suite, Version 3.11; AOPWIN Program, Version 1.91; PC-Computer software developed by EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC).
 Reliability: (Klimisch Code 2) Valid with restrictions, calculated value.

7.0 STABILITY IN WATER

Type:	Abiotic (hydrolysis) [X]; biotic (sediment) []
Half life:	2.0 h, 2.5 h, and 1.5 h at pH 2, pH 5-6, and pH 9, respectively.
Degradation:	Yes (See remarks below)
Method:	OECD Test Guideline 111 (2004)
GLP:	Yes [] No [X] ? []
Test Substance:	4,4'-Bisphenol A dianhydride (Bisphenol A Dianhydride; BPA-DA; CAS RN 38103-06-9); Lot UI0054 from General Electric Plastics; Purity: 98.2%.
Remarks:	With respect to OECD Guideline 111, BPD-DA was considered hydrolytically unstable at pH 4, 7 and 9 at 23°C. No information on the hydrolysis of BPA-DA was found in the literature. The hydrolysis of phthalic anhydride to phthalic acid is shown in the following figure (Andres et al., 2001; Fagley et al., 1969; Hawkins, 1975):



Since the BPA-DA molecule contains phthalic anhydride moieties as the only hydrolytically susceptible groups, the hydrolysis of BPA-DA is expected to proceed to first the diacid (2Ac) and finally to the tetraacid (4Ac), as illustrated in the following figure:

**Preparation of Solutions:**

pH 2 Solution: 1 N aqueous HCl (~ 2 mL) was added to 100 mL deionized water to pH 2.0.

Deionized water: Historical data: pH 5 to 6.

pH 9 Solution: 1 N aqueous NaOH (~ 1 drop) was added to 100 mL deionized water to pH 9.0.

Solution SkDA1 (1 mg/mL nominal BPA-DA concentration in CH_3CN solution): 4.95 mg BPA-DA was mixed with 5.00 mL CH_3CN .

Solution SkDA2 (BPA-DA solution in CH_3CN) (~ 0.5 mg/mL): ~ 5 mg BPA-DA was mixed with 10.00 mL CH_3CN . Minor amounts of BPA-DA remained undissolved.

The calibration curve from the Calibration Solutions showed a linear relationship with a correlation coefficient (R^2) of 0.9974, indicating acceptable method precision for the analysis of BPA-DA in acetonitrile solution. The detection limit was defined as the lowest concentration of an analyte that an analytical process can reliably detect. The Detection Limit (DL) was qualitatively estimated at 80 $\mu g/L$ (corresponding to 8 ng

of BPA-DA injected on-column) for the detection of BPA-DA in acetonitrile solution. Multiplying this DL by 5 gave a quantification limit (QL) of 400 µg/L.

Solutions of BPA-DA in acetonitrile were prepared, to which were added various volumes of deionized water (DIW, pH 5-6), acidic (pH 2) DIW, or basic (pH 9) DIW, to achieve aqueous concentrations ranging from 1 to 40 v/v %. The solutions were then directly analyzed by HPLC-UV. The chromatograms of the acidic, neutral and basic solutions at 1% BPA-DA showed the presence of BPA-DA, 2Ac and minor 4Ac peaks, while the 40% solutions showed only the 'terminal' hydrolysis product 4Ac. The results displayed decreasing BPA-DA and increasing 4Ac in solutions with increasing aqueous concentrations. The intermediate hydrolysis product 2Ac concentrations showed maxima at 10% aqueous solutions. These results were consistent with the hypothesized hydrolysis pathway (above). Using a first order kinetic model, the half-lives of BPA-DA in acetonitrile/aqueous (60/40, v/v) solutions were estimated at 1.5 h, 2.0 h and 2.5 hour for the basic, acidic, and neutral conditions, respectively, at 23°C.

Given that the above estimated BPA-DA half-lives were obtained at high acetonitrile cosolvent concentration, and the hydrolysis rate should be faster in the absence of cosolvent, BPA-DA should be considered hydrolytically unstable at pH 4, 7 and 9 at 23°C, based on the OECD stability criterion of half-life greater than 1 year at 25°C.

Reference:

Reimer, G.J. (2005). Unpublished Report No. RAA7213 1449-Hy entitled "Physical/chemical property of BPA-DA [CAS # 38103-06-9]: Hydrolytic Stability (OECD 111)", dated June 10, 2005 for General Electric Company, Pittsfield, MA, USA; from Vison SciTec Inc., Vancouver, BC, Canada.

Andres, G.O., Granados, A.K. and de Rossi, R.H. (2001). Kinetic Study of the Hydrolysis of Phthalic Anhydride and Aryl Hydrogen Phthalates. *Journal of Organic Chemistry*, 66(23), 7653-7657.

Fagley, T.F. and Oglukian, R.L. (1969). Solvolysis of phthalic anhydride in dioxane-water mixtures. *Journal of Physical Chemistry*, 73(5), 1438-1447.

Hawkins, M.D. (1975). Hydrolysis of phthalic and 3,6-dimethylphthalic anhydrides. *Journal of the Chemical Society, Perkin Transactions 2: Physical Organic Chemistry (1972-1999)*, (4), 282-284.

Reliability:

(Klimisch Code 1) Valid without restrictions.

8.0 TRANSPORT AND DISTRIBUTION BETWEEN ENVIRONMENTAL COMPARTMENTS, INCLUDING ESTIMATED ENVIRONMENTAL CONCENTRATIONS AND DISTRIBUTION PATHWAYS

8.1 THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)

Type:	Fugacity model level III
Media:	Other: air, water, soil, sediment
Method:	Calculated [X] Measured []
GLP:	Yes [] No [X] ? []
Test Substance:	1,3-Isobenzofurandione, 5,5'- (1-methylethylidene)bis(4,1-phenyleneoxy) bis- [Bisphenol A Dianhydride; BPA-DA; CAS RN 38103-06-9]
Value:	Air < 0.1% Water = 1.18% Soil = 37.2% Sediment = 61.6%
Remarks:	Default values were assumed for environmental compartment descriptions, dimensions, and properties, advective and dispersive properties. Chemical-specific input parameters were: molecular weight = 520.50 g/mol; melting point = 187.50°C; and boiling point of 314°C. Emissions were assumed to be equally to air, water and soil. Air: $t_{1/2} = 16.1$ hr; emissions = 1000 kg/hr Water: $t_{1/2} = 3600$ hr; emissions = 1000 kg/hr Soil: $t_{1/2} = 3600$ hr; emissions = 1000 kg/hr Sediment: $t_{1/2} = 14,400$ hr; emissions = 0 kg/hr
Reference:	U.S. EPA (U.S. Environmental Protection Agency). 2003. EPI Suite, Version 3.11; Level III Fugacity Model; PC-Computer software developed by EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC).
Reliability:	(Klimisch Code 2) Valid with restrictions, calculated value.

9.0 BIODEGRADATION

Type:	Aerobic [X]; Anaerobic []
Inoculum:	Adapted []; Non-adapted [X]
Concentration of the chemical:	10 mg/L Related to COD []; DOC []; Test substance [X]; or, 4.0 mg/L as ThOD
Medium:	Water []; Water-sediment []; Soil []; Sewage treatment []; Other [X]: Deionized water with nutrients
Contact time:	28 days at $19 \pm 0.5^\circ\text{C}$
Degradation:	-1.6%

Result: Readily biodeg. []; Inherently biodeg. []; Other [];
Under test condition no (significant) biodegradation observed [X]

Kinetic of test substance:

Day	% Degradation of BPA-DA	% Degradation of Reference Substance	% Degradation of Toxicity Control
0	0.0	0.0	0.0
4	-0.3	---	---
7	0.6	72.2	74.9
11	0.3	---	---
14	0.8	78.9	73.2
18	0.4	---	---
21	-0.6	82.7	76.7
24	-0.2	---	---
28	-1.6	59.5	62.2

Test substance did not pass out of the acclimation phase during the evaluation.

Reference substance: Sodium benzoate

Kinetic of control

substance: See Above

Degradation Products: Yes [] No [] Not measured [X]

Method (Year): OECD Test Guideline 301D (1992)

GLP: Yes [X] No [] ? []

Test Substance: 4, 4'-Bisphenol A dianhydride (Bisphenol A Dianhydride; BPA-DA; CAS RN 38103-06-9); Lot UI0054 from General Electric Plastics; Purity: 98.2%.

Remarks: Following is a summary of test conditions

Parameter	Test Condition
Test type	OECD 301D – Closed Bottle Test (non-renewal) Aerobic
Duration	28 days
Inoculum	Secondary effluent from Lulu Island Domestic Wastewater Treatment plant, Richmond, BC
Test Solution Temperature	19.8 to 20.9°C
O ₂ Determination Method	Dissolved oxygen concentration using an oxygen electrode
Nitrification Determination Method	Not determined based on results
Test vessel	300-mL BOD bottles
Test volume	300 mL
Replicates	Two
Aeration	None
Controls	1. Seeded control (inoculated blank) 2. Positive control (reference substance plus inoculum) 3. Toxicity control (reference substance, BPA-DA and inoculum)

Parameter	Test Condition
Nominal BPA-DA concentration	10 mg/L
Nominal Reference Substance concentration	2 mg/L
Criterion for Ready Biodegradability	≥60% degradation in 10-day window within 28 day test period

Mean Biological Oxygen Demand (mgO₂/mg test substance)

Day	BPA-DA	Reference Substance	Toxicity Control
0	0.00	0.00	0.00
4	-0.01	---	---
7	0.01	1.21	1.25
11	0.01	---	---
14	0.02	1.32	1.22
18	0.01	---	---
21	-0.01	1.38	1.28
24	0.00	---	---
28	-0.03	0.99	1.04

Reference: Kinnee, K. (2005). Unpublished Report No. TOX0445 entitled “Closed Bottle Test of 4, 4’-Bisphenol A Dianhydride (CAS RN: 38103-06-9)(OECD Guideline 301D)”, dated February 14, 2005, for General Electric Company, Pittsfield, MA, USA; from Vizon SciTec Inc., Vancouver, BC, Canada.

Reliability: (Klimisch Code 1) Valid without restrictions.

ECOTOXICOLOGICAL DATA

10.0 ACUTE/PROLONGED TOXICITY TO FISH

Type of Test: Static [] Semi-static [] Flow-through [] Other []
 Open-system [] Closed-system []

Species: Rainbow trout (*Oncorhynchus mykiss*) (Spring Valley Trout Hatchery, Langley, BC, Canada)

Exposure Period: 96 hours

Results: LC₅₀ (96h) > 116 mg DAH/L
 NOAEC = 116 mg DAH/L
 LOAEC > 116 mg DAH/L

Analytical Monitoring: Yes [] No [] ? []

Method: OECD Test Guideline 203 (1992)
 The OECD 203 Guideline was modified, due to the difficult nature of the test substance. Its hydrolysis product, 1,2-benzenedicarboxylic acid, 4,4'-[(1-methylethylidene)bis(4,1-phenyleneoxy)]bis-(9CI)2,2-bis[4-(3,4-dicarboxyphenoxy)phenyl]propane (DAH; CAS RN 38103-05-8), was

tested to assess its potential to cause acute toxicity to Rainbow trout (*Oncorhynchus mykiss*).

GLP: Yes [X] No [] ? []

Test Substance: 4,4'-Bisphenol A Dianhydride (Bisphenol A Dianhydride; BPA-DA; CAS RN 38103-06-9); Lot UI0054 from General Electric Plastics; Purity: 98.2%; hydrolyzed to: 1,2-benzenedicarboxylic acid, 4,4'-[(1-methylethylidene)bis(4,1-phenyleneoxy)]bis-(9CI)2,2-bis[4-(3,4-dicarboxyphenoxy)phenyl] propane (DAH).

Remarks: Test conditions: Rainbow trout used for testing were obtained as swim-up fry from a government certified hatchery. The fish were held in water of similar quality to that used in the tests a minimum of 7 days before testing. The fish were in good health and free from any apparent malformation prior to testing. They were fed a minimum of 24 hours prior to test start, but were not fed during the test. See the table below for test parameters.

The stock solution was prepared by adding BPA-DA with acetonitrile (CAN) to dilution water a minimum of 24 hours before its use to ensure complete hydrolysis of BPA-DA to DAH. For the test, each replicate at each exposure concentration was prepared by adding an appropriate aliquot of DAH stock solution to 15 L of dilution water with 0.1 mL/L ACN as a solvent.

Chemical parameters (temperature, dissolved oxygen, pH, and conductivity) were measured at each observation period (e.g. 0, 24, 48, 72 and 96-hours). General observations on behavior were made at test initiation and at 24-, 48-, 72- and 96-hours. Observations of visible abnormalities were also recorded. Fish were considered to be dead if there was no visible movement (e.g., gill movement), and if touching of the caudal peduncle produced no reaction. An LC50 (concentration causing lethality to 50% of the organisms) was estimated from the 96-hour mortality data.

The test was deemed valid if: i) mortality and impairment did not exceed 10% in the control, ii) constant conditions were maintained throughout the test, iii) the dissolved oxygen concentration was at least 60% of the air saturation value throughout the test, and iv) the concentration of the substance being tested was maintained (within 80% of nominal) throughout the test. A concurrent reference substance was not run; however, data from a separate study using reagent grade phenol as the reference substance was used to assess the relative sensitivity of the test organisms and the precision of data produced by the laboratory.

The following is a summary of test conditions:

Parameter	Test Condition
Test type	Static
Duration	96 hours
Test organism	Rainbow trout (<i>Oncorhynchus mykiss</i>) Kamloops strain
Source of test organisms	Spring Valley Trout Hatchery, 4788 232 nd Street, Langley, BC, Canada
Average weight \pm SD & range	1.41 \pm 0.17 g / 1.19 – 1.66 g
Average fork length \pm SD & range	5.1 \pm 0.2 cm / 4.6 – 5.4 cm
Loading density	0.85 g/L
Test organism arrival date	June 3, 2004
Fish batch identification	KV040603
Mortality in 7 days preceding test (%)	0.31
Photoperiod	8-h dark and 16-h light
Light intensity	100 - 500 lux at the water surface
Temperature	15.3 to 16.4 °C
Dissolved oxygen	7.1 to 9.9 mg/L (72% to 99% air-saturation)
Feeding	None
Test vessel	20-L glass aquaria
Test volume	15 L
Loading density	9 fish per test vessel
Replicates	One
Aeration	6.5 \pm 1 mL/L/min
Dilution water source	Dechlorinated city tap water, hardened with CaCO ₃
Dilution water hardness	21 mg/L as CaCO ₃
Dilution water alkalinity	22 mg/L as CaCO ₃
Dilution water pH	7.4
Controls	Dilution water Solvent (0.1 mL/L ACN)
Nominal concentrations	0, 8, 16, 32, 64, 128 mg DAH/L
Adjusted nominal concentrations	0, 7.78, 15.6, 31.1, 62.2 and 124 mg DAH/L
Criterion for effect	Death
Toxicity Endpoints	96-hour LC50

Concentrations of DAH were measured in the controls and all test solutions at test initiation and termination. The stock solution concentration was also measured at test initiation. Samples were analyzed by HPLC with UV detection.

Results:

Concentrations of DAH were measured in the controls and all test solutions at test initiation and termination. The 62.2 mg/L test solution at test termination was analyzed in duplicate and the relative difference between the two analyses was 1%. The average observed/nominal DAH

concentration ratio in the matrix spiked samples was $103 \pm 0\%$. Measured DAH concentrations were 78 to 96% of adjusted nominal DAH concentrations at test initiation and 71 to 95% of adjusted nominal DAH concentrations at test termination. Mean measured DAH concentrations were 75 to 94% of nominal DAH concentrations. The mean measured concentrations were used to calculate the test endpoints as the measured test solution concentrations were not within 80 to 120% of the adjusted nominal concentrations.

Measured DAH Concentrations in Test Solutions (mg/L)

Adjusted Nominal DAH Concentration	Test Initiation ^a	Test Termination ^a	Mean ^a
Control	<DL ^b	<DL	0
Solvent Control	<DL	<DL	0
7.78	6.09 (78%)	5.53 (71%)	5.81 (75%)
15.6	12.2 (78%)	11.9 (76%)	12.1 (77%)
31.1	28.6 (92%)	28.8 (93%)	28.7 (92%)
62.2	55.3 (89%) ^c	59.0 (95%)	57.2 (92%)
124	118 (96%)	114 (92%)	116 (94%)
15,562 (Stock)	16,040 (103%)	N/A	N/A

^a Measured concentrations as a percentage of the adjusted nominal concentrations are listed in parentheses.

^b The detection limit was 3 mg/L.

^c Mean of duplicate analysis (59.4 and 58.7 mg/L).

The toxicity test met the test validity criteria, as there was no mortality in the controls, constant conditions were maintained during the test, and the dissolved concentrations were >60% saturation. In addition, the water quality measurements (e.g. pH, dissolved oxygen) were within the tolerance limits of the test organisms. The separate study conducted with phenol indicated that the test organisms responded normally to the reference substance.

At 96-h, no fish had died in any of the treatment groups. All surviving fish appeared normal throughout the exposure period. The LC50, based on mean measured DAH concentrations, was >116 mg DAH/L. Based on visual inspection of the data, the NOAEC was 116 mg DAH/L, and the LOAEC was >116 mg DAH/L.

Reference: Kinnee, K.J. (2005). Unpublished Report No. TOX0444 entitled “Acute Toxicity Test of 4,4’-Bisphenol A Dianhydride Hydrolysis Product Using Rainbow Trout, *Oncorhynchus mykiss* (OECD Guideline 203)”, dated February 1, 2005, for General Electric Company, Pittsfield, MA, USA; from Vison SciTec Inc., Vancouver, BC, Canada..

Reliability: (Klimisch Code 1) Valid without restrictions.

11.0 TOXICITY TO AQUATIC PLANTS (E.G. ALGAE)

Species:	Green algae (<i>Selenastrum capricornutum</i>) Strain UTCC37
End-point:	Biomass [] Growth rate [X] Other []
Exposure Period:	96 hours
Results:	Growth: EC ₅₀ (96h) = 1713 DAH mg/L NOAEC < 877 DAH mg/L LOAEC = 877 DAH mg/L
Analytical Monitoring:	Yes [X] No [] ? []
Method:	OECD Test Guideline 201 (1984) The OECD 201 Guideline was modified, as the test was extended to 96-h, upon request of the Sponsor. In addition, due to the difficult nature of the test substance, its hydrolysis product, 1,2-benzenedicarboxylic acid, 4,4'-[(1-methylethylidene)bis(4,1-phenyleneoxy)]bis-(9CI)2,2-bis[4-(3,4-dicarboxyphenoxy)phenyl]propane (DAH; CAS RN 38103-05-8), was tested to assess its potential to cause acute toxicity to the green algae, <i>Selenastrum capricornutum</i> .
GLP:	Yes [X] No [] ? []
Test Substance:	4, 4'-Bisphenol A Dianhydride (Bisphenol A Dianhydride; BPA-DA; CAS RN 38103-06-9); Lot UI0054 from General Electric Plastics; Purity: 98.2%; hydrolyzed to: 1,2-benzenedicarboxylic acid, 4,4'-[(1-methylethylidene)bis(4,1-phenyleneoxy)]bis-(9CI)2,2-bis[4-(3,4-dicarboxyphenoxy)phenyl]propane (DAH).
Remarks:	<i>S. capricornutum</i> , strain UTCC 37, was obtained from the Department of Botany Culture Collection, the University of Toronto, Ontario, Canada as a sterile liquid starter culture and a sterile algal slant (Organism Lot #UT040225). These starter cultures were used to initiate new cultures of <i>Selenastrum</i> . The algae was cultured in 250-mL glass Erlenmeyer flasks, using sterile nutrient media (SNM) and according to the procedures in the Vison SOPs " <i>Selenastrum capricornutum</i> 72-h Growth Inhibition Test" and "OECD Alga, Growth Inhibition Test." The nominal DAH concentrations used in this test was 770, 1540, 3079, 6415, and 12830 mg/L. The dilution factor between the concentrations was 2.0, except between 2880 and 6000 mg/L, where the dilution factor was 2.1. These concentrations bracketed the approximate 72-h EC50 value estimated from the Range-Finding Test. The stock solution was prepared with BPA-DA on the day prior to test initiation to ensure complete hydrolysis to DAH occurred. After preparation, the stock solution was stored in the dark at 4 ± 2°C. Dilution water with a concentration of 0.1 mL/L ACN was prepared a day prior to initiation for use in the test solution preparation. On the day of test initiation, a total of 500 mL of each test concentration was prepared by adding an aliquot of the appropriate DAH stock solution to the dilution water containing 0.1 mL/L ACN. The test vessels were pre-conditioned with each test solution. Each test solution was

thoroughly mixed before dispensing a 50 mL aliquot into each of the test vessels.

Two sets of negative controls, one with only dilution and one with 0.1 mL/L ACN and dilution water, were prepared. The controls were treated the same as the test solutions, as outlined above.

The temperature of the test chamber was monitored daily using a min/max thermometer. The pH of the test solutions was determined at test initiation and test termination and conductivity was measured at test initiation.

At 24-, 48-, 72- and 96-h, a minimum of four cell counts were performed on aliquots (10 μ L) from each flask, using a Bright-Line haemocytometer (Hausser Scientific, Horsham, PA). An average of four counts (10 μ L volume) was multiplied by 10^4 to estimate the cell concentration in each replicate (cells/mL). Additional counts were made in some of the test concentrations when the four counts varied excessively. On the haemocytometer, 25 squares were counted. In all counts, cells touching the outer top or left border were not counted, whereas, cells touching the outer bottom or right border were counted. The average of these counts was used as the cell yield for that flask.

The mean cell concentration was plotted against time for each test concentration, including the controls to examine the effect of DAH on the growth curves. The measured number of cells/mL at time was calculated by taking the average of the counts from each replicate flask during each counting period. The area under the growth curves (AUGC) for exponentially growing cultures was determined. The concentration-response relationship was examined by plotting the AUGC and mean AUGC values vs. mean measured concentration of DAH. This plot was used to select an appropriate model for non-linear regression analysis to fit the experimental data and determine the 96-h EC50. This plot was also used to estimate initial values for model parameters.

The non-linear regression function was used to estimate the 96-h EC50 and 95% confidence limits. The no-observed-adverse-effect-concentration, NOAEC, and the lowest-observed-adverse-effect concentration, LOAEC, were determined by one-way analysis of variance (ANOVA) and Dunnett's multiple comparison test performed using SAS JMP 4.0.2 (SAS Institute 2000). The mean measured DAH concentrations were used to calculate the test endpoints, as the measured concentrations were not within 80 to 120% of the adjusted nominal concentrations.

For the results of a growth inhibition test to be acceptable and the test to be considered valid, the following conditions must be satisfied: the number of alga cells in the standard controls must have increased by a factor of at least 16 in 72 h; and the disappearance of the test substance from the water into the algal biomass does not necessarily invalidate the test.

A test with a concurrent reference substance test was not performed, however, a reference substance test, with zinc sulphate heptahydrate, was

conducted as a separate study to assess the relative sensitivity of the test organisms and the precision of data produced by the laboratory.

The following is a summary of the test conditions:

Parameter	Test Conditions
Test Type	Static
Duration of test	96 hours
Test species	<i>Selenastrum capricornutum</i> , strain UTCC 37
Source of test organisms	Dept. of Botany Culture Collection, Univ. of Toronto, ON
Test organism arrival date	February 25, 2004
Test organism identification	UT040225
Culture medium	Sterile nutrient media
Testing medium	Sterile nutrient media
Temperature	23 ± 2°C
Photoperiod	24 hours
Light intensity	8000 ± 20%
Test vessels	250-mL Erlenmeyer flasks
Test solution volume	50 mL
pH of the test solutions	6.9 - 7.7
Age of test plants	3 days
Number of cells per test vessel	0.97 x 10 ⁴ cells/mL
Controls	Negative control (dilution water only) Solvent control (0.1 mL/L ACN)
Nominal DAH concentrations	770, 1540, 3079, 6415 and 12830 mg DAH/L
Adjusted nominal DAH concentrations	767, 1533, 3066, 6388 and 12777 mg DAH/L
Replicates	6 for the controls, 3 for each DAH concentration
Measured water quality parameters	Conductivity at study initiation pH at study initiation and termination
Measured endpoints	Number of cells
Calculated endpoints	EC50 at 96-h
Test validity criteria	Number of alga cells in the controls must have increased by a factor of at least 16 in 72 hours.

Concentrations of DAH were measured in the controls and all test concentrations at test initiation and termination. The stock solution concentration was also measured at test initiation. Samples were analyzed by HPLC with UV detection.

Results:

The 1,533 and 6,388 mg DAH/L test solutions were analyzed in duplicate and the relative difference between the two analyses were 0.5 and 2%, respectively. The average observed/nominal DAH concentration ratio in the matrix spiked samples was 101 ± 0 %. Measured DAH concentrations were 97 to 103% of adjusted nominal DAH concentrations at test initiation and 109 to 131% of nominal DAH concentrations at test termination. The measured concentrations were higher at test termination

than at test initiation. Mean measured DAH concentrations were 105 to 115% of nominal DAH concentrations.

Measured DAH Concentrations in Test Solutions (mg/L)

Adjusted Nominal DAH Concentration	Test Initiation ^a	Test Termination ^a	Mean ^a
Control	<DL ^b	<DL	0
Solvent Control	<DL	<DL	0
767	752 (98%)	1001 (131%)	877 (114%)
1533	1546 (101%)	1665 (109%) ^c	1606 (105%)
3066	3155 (103%)	3421 (112%)	3288 (107%)
6388	6367 (100%)	8310 (130%) ^d	7339 (115%)
12777	12,846 (101%)	14808 (116%)	13827 (108%)
25553 (Stock)	24,700 (97%)	N/A	N/A

^a Measured concentrations as a percentage of the adjusted nominal concentrations are listed in parentheses.

^b The detection limit was 3 mg/L.

^c Mean of duplicate analysis: 1673 and 1657 mg/L.

^d Mean of duplicate analysis: 8452 and 8168 mg/L.

The cell counts in each 24-h period during the test and the mean AUGC data are summarized in the following table. The concentration-response data was examined by plotting the AUGC versus concentration based on mean measured DAH concentrations. The mean measured DAH concentrations were used for the test endpoint calculations because the measured concentrations were not within 80 to 120% of nominal concentrations.

Adjusted Nominal DAH Concentration (mg/L)	Mean Measured DAH Concentration (mg/L)	Average Cell Counts ($\times 10^4$ /mL)					Mean Area Under the Growth Curve
		0-h	24-h	48-h	72-h	96-h	
Control	0	0.97	3.5	18.0	149.0	179.8	6168
Solvent Control	0	0.97	3.9	18.8	154.8	193.1	6492
767	877	0.97	3.2	15.4	123.4	205.3	5790
1533	1606	0.97	3.1	15.3	85.9	108.9	3729
3066	3288	0.97	1.7	5.8	17.5	31.8	897
6388	7339	0.97	0.8	1.9	0.8	1.6	21
12777	13827	0.97	1.2	0.5	0.7	0.8	-17

The toxicity test met the test validity, as the number of algae cells in the dilution water controls increased by a factor of 151 in 72 h. The pH values were within 1 pH unit at test initiation and termination and were within of the tolerance limits (6.5 to 8.5) of the test organisms. There was a slight hormetic effect (stimulation) on algal growth observed in the solvent control, as compared to the dilution water control. The separate

study conducted with zinc sulphate heptahydrate indicated that the test organisms responded normally to the reference substance.

The 96-h EC50 and 95% confidence limits, based on mean measured DAH concentrations, were 1713 mg DAH/L (1420 – 2086). The NOAEC was <877 mg DAH/L and the LOAEC was 877 mg DAH/L.

Reference: Kinnee, K. (2003). Unpublished Report No. TOX0442 entitled “Growth Inhibition Test of 4,4’-Bisphenol A Dianhydride Hydrolysis Product Using *Selenastrum capricornutum* (OECD Guideline 201)”, dated February 3, 2003, for General Electric Company, Pittsfield, MA, USA; from Vison SciTec Inc., Vancouver, BC, Canada.

Reliability: (Klimisch Code 1) Valid without restrictions.

12.0 ACUTE TOXICITY TO AQUATIC INVERTEBRATES (E.G. DAPHNIA)

Type of Test: Static Semi-static Flow-through Other
 Open-system Closed-system

Species: *Daphnia magna*

Exposure Period: 96 hours

Results: EC₅₀ (96h) > 1248 mg DAH/L
 NOEC = 1248 mg DAH/L
 LOEC > 1248 mg DAH/L

Analytical Monitoring: Yes No ?

Method: OECD Test Guideline 202 (1984)
 The OECD 202 Guideline was modified, as the test was extended to 96-h, upon request of the Sponsor. In addition, due to the difficult nature of the test substance, its hydrolysis product, 1,2-benzenedicarboxylic acid, 4,4'-[(1-methylethylidene)bis(4,1-phenyleneoxy)]bis-(9CI)2,2-bis[4-(3,4-dicarboxyphenoxy)phenyl]propane (DAH), was tested to assess its potential to cause acute toxicity to *Daphnia magna*.

GLP: Yes No ?

Test Substance: 4,4’-Bisphenol A Dianhydride (Bisphenol A Dianhydride; BPA-DA; CAS RN 38103-06-9); Lot UI0054 from General Electric Plastics; Purity: 98.2%; hydrolyzed to: 1,2-benzenedicarboxylic acid, 4,4'-[(1-methylethylidene)bis(4,1-phenyleneoxy)]bis-(9CI)2,2-bis[4-(3,4-dicarboxyphenoxy)phenyl]propane (DAH).

Remarks: Test conditions: The *Daphnia* cultures were originally obtained from Pacific Environmental Science Centre, North Vancouver, Canada and then maintained in an in-house culture. Prior to the test, the *Daphnia* cultures were fed 2.8 mL each of green algae (*Chlorella* sp. and *Selenastrum capricornutum*) and yeast-cereal-grass fermented-trout-chow (YCT) three times per week. The neonates used in the test were fed 6 mL of concentrated *Chlorella* sp. and 6 mL of concentrated *S. capricornutum* on the day of test initiation. The algae cultures were obtained from the University of Toronto Culture Collection, Toronto, ON. The yeast, Fleischmann’s Traditional Dry Active Yeast, was obtained from Fleischmann’s Yeast, LaSalle, QC; the cereal grass, Ward’s Cereal Grass Media, was obtained from Ward’s Natural Science, St. Catherine’s, ON,

Canada; and trout chow, Nutra-Plus 0 Crumble, was obtained from Skretting, Vancouver, BC, Canada.

To reduce adsorption losses, all glassware used in this study was pre-conditioned with the appropriate test solution prior to use.

The concentrations used in the test were chosen based on the results of a range-finding test conducted previously. The OECD Guideline states that the highest concentration in a definitive test should not exceed 1000 mg/L, however, the measured DAH concentration of the highest concentration tested in the range-finding test (nominal 1283 mg DAH/L) was 866 mg/L. Therefore, to compensate for lower than expected concentration of the hydrolysis product, a slightly higher (1283 mg DAH/L) concentration than the 1000 mg/L limit was tested to ensure that the concentration of hydrolysis product was within 20% of the 1000 mg/L limit.

Stock solutions were prepared with BPA-DA on the day prior to test initiation to ensure complete hydrolysis to DAH occurred. After preparation, the stock solution was stored in the dark at $4 \pm 2^\circ\text{C}$.

The pH of the lowest concentration (78 mg DAH/L) was 7.9 and was within the pH tolerance range (6.0 to 8.5) of the test organisms; therefore, it was not necessary to prepare any lower concentrations.

The test solutions were renewed on Day 2. Approximately 3 h prior to test solution renewal, 80 μL each of *Chlorella*, *Selenastrum* and YCT were added to each test vessel. The test solutions were prepared as outlined above for Day 0, using the previously prepared stock solution and the previously used glassware. The *Daphnia* were temporarily transferred from the test solutions into glass vials, the test solutions were replaced and the *Daphnia* were transferred into the freshly prepared test solutions using a glass, wide-bore pipette.

Conductivity was measured at test initiation and in the freshly prepared solutions on Day 2. The temperature, pH, and dissolved oxygen (DO) concentrations were measured in the test solutions at test initiation, before and after renewal on Day 2, and at test termination. Initial measurements were taken from a separate test vessel to avoid contamination of the test solutions. This test vessel was filled with the same solutions and treated as the other test vessels. Interim and final measurements were taken in each replicate.

General observations on behavior, such as immobilization, were made and recorded at test initiation and at 24-, 48-, 72- and 96-h of exposure. Immobilization was defined as the inability to swim during a period of 15 sec following gentle agitation of the test solution. The test was terminated at 96-h.

The following is a summary of test conditions:

Parameter	Test Condition
Test type	Static renewal
Duration of test	96 hours
Test organism	<i>Daphnia magna</i>
Age at Test Initiation	< 25-h
Source of Test Organisms	In-house culture; original culture obtained November 2001 from Pacific Environmental Science Centre, North Vancouver, Canada
Test Organism ID	PE040713
Room Temperature	19-21°C
Test Solution Temperature	19.3 - 20.9°C
Photoperiod	16-hour light, 8-hour dark
Light intensity	400 - 800 lux
Dissolved Oxygen Concentration	8.6 to 9.2 mg/L (96 to 101% saturation)
Feeding	80 µL each replicate of YCT, <i>Selenastrum</i> and <i>Chlorella</i> on Day 2, prior to water change
Test vessel	250 mL borosilicate beakers
Test volume	200 mL
Replicates	Four
Number of Test Organisms per Replicate	Five
Aeration	None; the test vessels were covered with Plexiglas sheets during the exposure period
Dilution Water Source	Moderately hard, reconstituted water
Dilution Water Hardness	95 mg CaCO ₃ /L (Test Initiation) 98 mg CaCO ₃ /L (Renewal Day 2)
Dilution Water Alkalinity	65 mg CaCO ₃ /L (Test Initiation) 64 mg CaCO ₃ /L (Renewal Day 2)
Dilution Water pH	8.0 (Test Initiation) 7.8 (Renewal Day 2)
Dilution Water Ca/Mg Molar Ratio	1.1 (Test Initiation) 1.0 (Renewal Day 2)
Dilution Water Na/K Molar Ratio	20.9 (Test Initiation) 959 ^a (Renewal Day 2)
Controls	Negative Control (dilution water only) Solvent Control (0.1 mL/L ACN)
Nominal DAH concentrations	80, 160, 321, 641 and 1283 mg DAH/L
Adjusted Nominal DAH concentrations	78, 156, 312, 624 and 1248 mg DAH/L
Criterion for effect	Immobilization
Calculated toxicity values	EC ₅₀ at 96-h

^a The measured K concentration of the *Daphnia* water prepared for renewal on Day 2 was considerably less than the nominal concentration. Therefore, the Na/K molar ratio was significantly higher than that of the water prepared for test initiation.

The adjusted nominal DAH concentrations were used to calculate the test endpoints, as the measured concentrations were within 80 to 120% of the adjusted nominal concentrations.

The test validity conditions were: in the control, not more than 10% of the *Daphnia* should be immobilized; and the dissolved oxygen concentrations at the end of the test should be $\geq 60\%$ of the air saturation at the temperature used.

Concentrations of DAH were measured in the control and all test solutions at test initiation, before and after renewal on Day 2, and at test termination. The stock solution concentration was also measured at test initiation and before renewal on Day 2. The samples were analyzed by HPLC with UV detection.

A concurrent reference substance test was not performed, however, a reference substance test, with zinc sulphate heptahydrate, was conducted as a separate study to assess the relative sensitivity of the test organisms and the precision of data produced by the laboratory.

Results:

The 312 and 1248 mg/L test solutions were analyzed in duplicate and the relative difference between the two analyses were 1 and 0%, respectively. The average observed/nominal DAH concentration ratio in the matrix spiked samples was $101 \pm 0\%$. Measured DNA concentrations were 97 – 101%, 96 – 101%, 99 – 103% and 99 – 105% of the adjusted nominal DAH concentrations at test initiation, on Day 2 (before), on Day 2 (after) and at test termination, respectively. Mean measured DAH concentrations were 98 – 102% of adjusted nominal DAH concentrations.

Adjusted Nominal DAH Concentration	Test Initiation ^a	Day 2 Before ^a	Day 2 After ^a	Test Termination ^a	Mean ^a
Control	<DL ^b	<DL	<DL	<DL	0
Solvent Control	<DL	<DL	<DL	<DL	0
78	75.3 (97%)	74.5 (96%)	78.4 (100%)	77.8 (100%)	76.5 (98%)
156	155 (99%)	153 (98%)	155 (99%)	154 (99%)	154 (99%)
312	316 (101%) ^c	314 (101%)	312 (100%)	320 (103%)	316 (101%)
624	606 (97%)	608 (97%)	624 (100%)	628 (101%)	617 (99%)
1248	1251 (100%)	1250 (100%)	1271 (102%) ^d	1309 (105%)	1270 (102%)
10,403 (Stock)	10,405 (100%)	N/A	10,739 (103%)	N/A	10,572 (102%)

^a Measured concentrations as a percentage of the adjusted nominal concentrations are listed in parentheses.

^b The detection limit was 3 mg/L.

^c Mean of duplicate analysis: 314 and 318 mg/L.

^d Mean of duplicate analysis: 1271 and 1270 mg/L.

The 48-h LC50 and 95% confidence intervals for the reference substance test were within the acceptable range (± 2 SD) of previous tests conducted in this laboratory. These results indicate that the test organisms were responding normally to the reference substance. The toxicity test met the test validity criteria, because there were no immobilized organisms in the controls, and because the dissolved oxygen concentrations at the test termination were $>60\%$ saturation. The water quality measurements (e.g.

dissolved oxygen, pH) were also within the tolerance limits of the test organism.

After the 96-h exposure, there was one *Daphnia* (5%) immobilized at 624 mg/L and 7 *Daphnia* (35%) immobilized at 1248 mg DAH/L. Therefore, the 96-h EC50 estimate was >1248 mg DAH/L, based on adjusted nominal concentrations. The NOEC and LOEC were 1248 mg DAH/L and >1248 mg DAH/L, respectively.

Reference: Kinnee, K. J. (2005). Unpublished Report No. TOX0443 entitled “Acute Immobilisation Test of 4,4’-Bisphenol A Dianhydride Hydrolysis Product using *Daphnia magna* (OECD Guideline 202)”, dated February 2, 2005, for General Electric Company, Pittsfield, MA, USA; from Vizion SciTec Inc., Vancouver, BC, Canada..

Reliability: (Klimisch Code 1) Valid without restrictions.

TOXICITY

13.0 ACUTE TOXICITY

13.1 ACUTE ORAL TOXICITY

13.1.1

Type: LD₀ [] LD₁₀₀ [] LD₅₀ [X] LD_{Lo} [] Other []

Species/Strain: Rat/Sprague-Dawley

Sex: Male and female

Animals: 5/sex

Vehicle: Distilled water

Value: > 5000 mg/kg

Method: US EPA Toxic Substance Control Act (TSCA) Health Effects Test Guidelines (1982 and 1984)

GLP: Yes [] No [X] ? []

Test Substance: Biphenol Dianhydride (Bisphenol A Dianhydride; BPA-DA; CAS RN 38103-06-9); from General Electric Company; Purity: >99%.

Remarks: An appropriate amount of the test substance was mixed with distilled water to give a 50% concentration. The resulting suspension was administered to five male and five female rats at a dose volume of 10 mL/kg which provided a dose of 5000 mg/kg of biphenol dianhydride. The dose was administered by gavage through a commercial 16 gauge (3-inch) ball-end stainless steel needle attached to a disposable syringe. The rats were fasted from food overnight (approximately 18 hours) before dosing. The rats weighed between 200 and 250 g and were approximately 5 to 8 weeks of age at the time of dosing. Animals were observed for signs of toxic effects frequently on the day of dosing and twice daily thereafter for 14 days. Body weights were recorded on the day of dosing and at 7 and 14 days after dosing. A gross necropsy was performed on all animals 14 days after dosing.

Results: All rats survived and gained weight during the 14-day post-dosing period. There were no signs of toxicity during the 14-day post-dosing observation

	period and there were no remarkable gross pathologic lesions found at necropsy.
Reference:	Myers, R. C. (1988). Unpublished report for Project No. 50-667 entitled “Biphenol Dianhydride Acute Toxicity and Primary Irritancy Studies”, dated January 19, 1988 for General Electric Company, Pittsfield, MA, USA; from Bushy Run Research Center, Export, PA, USA.
Reliability:	(Klimisch Code 1) Valid without restrictions – Guideline study.
13.1.2	
Type:	LD ₀ [] LD ₁₀₀ [] LD ₅₀ [] LD _{Lo} [] Other [X]
Species/Strain:	Rat/Spartan (albino)
Sex:	Male
# Animals:	4
Vehicle:	Corn oil
Value:	Not applicable
Method:	Not specified
GLP:	Yes [] No [X] ? []
Test Substance:	AR No. 82896 (Bisphenol A Dianhydride; BPA-DA; CAS RN 38103-06-9); from General Electric Company; Purity: See “Chemical Identity and Use Information” section.
Remarks:	Rats weighed from 220 to 234 grams at the initiation of the study. Animals were fasted from food overnight prior to dosing. The test material was administered to two rats each at doses of 500 and 5000 mg/kg. The test compound was suspended in corn oil at concentrations enabling the administration of 10 mL/kg at the 500 mg/kg dose and 20 mL/kg at the 5000 mg/kg dose. All rats were observed for mortality for a period of 14 days. Body weights were measured initially and at 14 days.
Results:	All rats survived the 14-day observation period and exhibited normal body weight gains.
Reference:	Wazeter, F. X. and Goldenthal, E. I. (1974). Unpublished report for Project No. 313-035 entitled “Acute Toxicity Screening Studies in Rats and Rabbits”, dated May 16, 1974 for General Electric Company, from International Research and Development Corporation, Mattawan, MI, USA.
Reliability:	(Klimisch Code 2) Valid with restrictions. Only two animals dosed per dosage group.

13.2 ACUTE INHALATION TOXICITY

No studies were found.

13.3 ACUTE DERMAL TOXICITY

13.3.1

Type: LD₀ [] LD₁₀₀ [] LD₅₀ [X] LD_{L0} [] Other []
 Species/Strain: Rabbit/New Zealand White
 Value: > 2000 mg/kg
 Method: US EPA Toxic Substance Control Act (TSCA) Health Effects Test Guidelines (1982 and 1984)
 GLP: Yes [] No [X] ? []
 Test Substance: Biphenol Dianhydride (Bisphenol A Dianhydride; BPA-DA; CAS RN 38103-06-9); from General Electric Company; Purity: >99%.
 Remarks: Ten rabbits (5/sex), weighing 2.0 to 3.0 kg and approximately 12 to 18 weeks of age, were dosed with a single dermal application of 2000 mg biphenol dianhydride/kg body weight for 24 hours. The test substance was moistened with distilled water and applied to the dorsal surface of the clipped trunk of each rabbit. The coverage was approximately 43 mg of sample per cm² of rabbit skin surface. A double layer of gauze sheeting was wrapped around the trunk and secured with adhesive tape. Polyethylene sheeting and bandaging tape were then wrapped around the trunk. Animals were returned to their cages. After 24 hours, all covering was removed and as much excess test material as possible was carefully removed. Treated rabbits were observed frequently for signs of toxic effects on the first day of the test and twice daily thereafter for 14 days.
 Results: All rabbits survived and there were no signs of toxicity during the test. All rabbits gained weight during the 14-day post-application period. No local skin reactions were apparent. Gross pathologic evaluation revealed only a pitted surface of the kidneys of one male.
 Reference: Myers, R. C. (1988). Unpublished report for Project No. 50-667 entitled "Biphenol Dianhydride Acute Toxicity and Primary Irritancy Studies", dated January 19, 1988 for General Electric Company, Pittsfield, MA, USA; from Bushy Run Research Center, Export, PA, USA.
 Reliability: (Klimisch Code 1) Valid without restrictions. Guideline study.

13.3.2

Type: LD₀ [] LD₁₀₀ [] LD₅₀ [] LD_{L0} [] Other [X]
 Species/Strain: Rabbit/New Zealand White
 Value: Not applicable
 Method: Not specified
 GLP: Yes [] No [X] ? []
 Test Substance: AR No. 82896 (Bisphenol A Dianhydride; BPA-DA; CAS RN 38103-06-9); from General Electric Company; Purity: See "Chemical Identity and Use Information" section.

Remarks:	Two New Zealand White rabbits (one male and one female) were used at each of two doses. The rabbits weighed from 2413 to 2729 grams at study initiation. Body weights were measured initially and at 14 days. The compound was applied to the clipped back of each rabbit. Two rabbits received 200 mg of the test substance/kg body weight and two rabbits received 2000 mg of the test substance/kg body weight. The application area was wrapped with a gauze bandage and occluded with plastic wrap. After 24 hours, the bandages were removed and the application areas were washed with tepid tap water. The rabbits were observed for mortality for a period of 14 days.
Results:	All rabbits survived the 14 day observation period. Three of the rabbits exhibited body weight gains and one rabbit showed a slight (81 gram) loss in body weight during the 14-day observation period.
Reference:	Wazeter, F. X. and Goldenthal, E.I. (1974). Unpublished report for Project No. 313-035 entitled “Acute Toxicity Screening Studies in Rats and Rabbits”, dated May 16, 1974 for General Electric Company, from International Research and Development Corporation, Mattawan, MI, USA.
Reliability:	(Klimisch Code 2) Valid with restrictions. Only two rabbits exposed per dose.

14.0 GENETIC TOXICITY *IN VITRO* OR *IN VIVO* (CHROMOSOMAL ABERRATIONS)

Type:	<i>In vitro</i> mammalian chromosome aberration test		
System of testing:	Chinese hamster ovary (CHO) cells		
Concentration:	Up to 1500 µg/mL without activation Up to 750 µg/mL with activation		
Metabolic activation:	With []; Without []; With and Without [X]; No data []		
Results:	Negative with and without metabolic activation		
Cytotoxicity conc:	With metabolic activation:	≥ 350 µg/mL	
	Without metabolic activation:	None (4 hr exposure)	
	Without metabolic activation:	≥ 500 µg/mL (20 hr exposure)	
Precipitation conc:	With metabolic activation:	≥ 250 µg/mL	
	Without metabolic activation:	≥ 250 µg/mL	
Genotoxic effects:		+	? -
	With metabolic activation:	[]	[] [X]
	Without metabolic activation:		
	4-hr Exposure:	[]	[] [X]
	20-hr Exposure:	[]	[] [X]
Method:	OECD Test Guideline 473 (1998)		
GLP:	Yes [X] No [] ? []		
Test Substance:	Bisphenol A Dianhydride (BPA-DA; CAS RN 38103-06-9); Lot UI0054; from General Electric Plastics; Purity: 98.2%.		
Remarks:	<u>Description of test procedure:</u> A preliminary toxicity assay was performed for the purpose of selecting doses for the chromosome aberration assay and consisted of an evaluation of test article effect on cell growth. CHO cells were seeded for each treatment condition at approximately 5x10 ⁵ cells/25 cm ² flask and were incubated at 37±1°C in a		

humidified atmosphere of $5\pm 1\%$ CO₂ in air for 16-24 hours. Treatment was carried out by refeeding the flasks with complete medium McCoy's 5A medium supplemented with 10% fetal bovine serum (FBS), 100 units penicillin and 100 µg streptomycin/mL, and 2 mM L-glutamine) for the non-activated study or S9 reaction mixture for the activated study, to which was added 50 µL dosing solution of test article in solvent or solvent alone. The osmolality in treatment medium of the solvent and of the highest test article concentration, the lowest precipitating test article concentration and the highest soluble test article concentration were measured. The pH of the highest concentration of dosing solution in the treatment medium was measured using test tape. The cells were treated for 4 hours with and without S9, and continuously for 20 hours without S9. At completion of the 4-hour exposure period, the cells were washed with buffered saline, refeed with complete medium and returned to the incubator for a total of 20 hours from the initiation of treatment. At 20 hours after the initiation of treatment, the cells were harvested by trypsinization 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. In the preliminary toxicity assay, the maximum dose tested was 2000 µg/mL. The test article formed a workable suspension in DMSO at a concentration of 2000 µg/mL, the maximum concentration prepared in the assay. Visible precipitate was observed in treatment medium at dose levels ≥ 200 µg/mL. Dose levels ≤ 60 µg/mL were soluble in treatment medium. The osmolality of the test article concentrations in treatment medium were acceptable because they did not exceed the osmolality of the solvent by more than 20%. The pH of the highest concentration of test article in treatment medium was approximately 7.0. Based on the toxicity study, the doses chosen for the chromosome aberration assay were: 0, 62.5, 125, 250, 500, 750, 1000, 1250 and 1500 µg/mL (4-hr treatment w/o activation); 0, 62.5, 125, 250, 500, 750, 1000, 1250 and 1500 µg/mL (20-hr treatment w/o activation); and 0, 62.5, 125, 250, 350, 500, 600 and 750 µg/mL (4-hr treatment with activation). Samples were run in duplicate, with and without metabolic activation.

Selection of doses for microscopic analysis: The first criterion, specified by the Guideline, is to select the highest dose with at least 50% reduction in cell growth or mitotic index relative to the solvent control with a sufficient number of scorable metaphase cells, regardless of test article precipitation in the treatment medium. For this study, the mitotic index was used to select the highest dose for each test condition. Two lower doses also were included.

For the chromosome aberration assay, CHO cells were seeded and treated as described above. The osmolality in treatment medium of the solvent and of the highest test article concentration, the lowest precipitating test article concentration and the highest soluble test article concentration

were measured. The pH of the highest concentration of dosing solution in the treatment medium was measured using test tape. A concurrent toxicity test was conducted for each treatment. After cell harvest the cells were counted, test article precipitate was assessed and cell viability was determined by trypan blue dye exclusion. Cell counts and viability were used to determine cell growth inhibition.

The cells were exposed to the test article continuously for 4 or 20 hours in the non-activated study, and for 4 hours in the activated study. After the exposure period for the 4-hour exposure groups, the cells were washed and returned to the incubator. Two hours prior to the scheduled cell harvest, Colcemid[®] was added to duplicate flasks for each treatment condition. Two hours after the addition of Colcemid[®], metaphase cells were harvested. Cells were collected approximately 20 hours after initiation of treatment. Slides were prepared from cells of each treatment and the cells stained with 5% Giemsa.

Evaluation of metaphase cells: The percentage of cells in mitosis per 500 cells scored (mitotic index) was determined for each treatment group. Initially, the non-activated and S9 activated 4-hour exposure groups were evaluated for chromosome aberrations and since a negative result was obtained in the non-activated 4-hour exposure group, the non-activated 20-hour continuous exposure group was then also evaluated for chromosome aberrations. When 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 reached 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 dicentric 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. 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. Statistical analysis of the percent aberrant cells was performed using the Fisher's exact test. Fisher's exact 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 exact test at any test article dose group, the Cochran-Armitage test was used to measure dose-responsiveness. 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 ($p \leq 0.05$). However, values that are statistically significant but do not exceed the range of historic solvent controls may be judged as not biologically significant. Test articles not demonstrating a statistically significant increase in aberrations will be concluded to be negative.

Criteria for a Valid Test: 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.

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 final concentrations of 10 and 20 $\mu\text{g/mL}$. The solvent vehicle for the test article, dimethyl sulfoxide (DMSO), was used as the solvent control at the same concentration as that found in the test article-treated groups.

Results:

In the chromosome aberration assay, visible precipitate was observed in treatment medium at dose levels $\geq 250 \mu\text{g/mL}$. Dose levels $\leq 125 \mu\text{g/mL}$ were soluble in treatment medium. The osmolality in treatment medium of the highest concentration tested, 1500 $\mu\text{g/mL}$, was 396 mmol/kg. The osmolality in treatment medium of the lowest precipitating concentration, 250 $\mu\text{g/mL}$, was 405 mmol/kg. The osmolality in treatment medium of the highest soluble concentration, 125 $\mu\text{g/mL}$, was 402 mmol/kg. The osmolality of the solvent (DMSO) in treatment medium was 398 mmol/kg. The osmolality of the test article concentrations in treatment medium are acceptable because they did not exceed the osmolality of the solvent by more than 20%. The pH of the highest concentration of test article in treatment medium was approximately 7.0.

4 hours in the absence of S9 activation: Toxicity of BPA-DA (cell growth inhibition relative to the solvent control) in CHO cells when treated for

4 hours in the absence of S9 activation was 17% at 500 µg/mL, the highest test concentration evaluated for chromosome aberrations. The mitotic index at the highest dose level evaluated for chromosome aberrations, 500 µg/mL, was 54% reduced relative to the solvent control. The dose levels selected for microscopic analysis were 125, 250 and 500 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 at any dose level ($p > 0.05$, Fisher's exact test). The percentage of structurally damaged cells in the MMC (positive control) treatment group (19.0%) was statistically significant.

4 hours in the presence of S9 activation: Toxicity of BPA-DA in CHO cells when treated for 4 hours in the presence of S9 activation was 52% at 350 µg/mL, the highest test concentration evaluated for chromosome aberrations. The mitotic index at the highest dose level evaluated for chromosome aberrations, 350 µg/mL, was 52% reduced relative to the solvent control. The dose levels selected for microscopic analysis were 125, 250 and 350 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 at any dose level ($p > 0.05$, Fisher's exact test). The percentage of structurally damaged cells in the CP (positive control) treatment group (21.0%) was statistically significant.

20 hours without of S9 activation: Toxicity of BPA-DA was 39% at 500 µg/mL, the highest test concentration evaluated for chromosome aberrations in the non-activated 20 hour continuous exposure group. The mitotic index at the highest dose level evaluated for chromosome aberrations, 500 µg/mL, was 51% reduced relative to the solvent control. The dose levels selected for microscopic analysis were 125, 250 and 500 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 at any dose level ($p > 0.05$, Fisher's exact test). The percentage of structurally damaged cells in the MMC (positive control) treatment group (20.0%) was statistically significant.

Conclusion:

The positive and solvent controls fulfilled the requirements for a valid test. Under the conditions of the assay, Bisphenol A Dianhydride (BPA-DA) was concluded to be negative for the induction of structural and numerical chromosome aberrations in CHO cells.

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 (%)	Cells with Structural Aberrations (%)
Vehicle (DMSO)	-	4	8.9	200	0.000 ± 0.000	1.5	0.0
125 BPA-DA	-	4	8.0	200	0.005 ± 0.071	2.5	0.5
250 BPA-DA	-	4	6.7	200	0.000 ± 0.000	3.0	0.0
500 BPA-DA	-	4	4.1	200	0.005 ± 0.071	4.5	0.5
Positive control (MMC) 0.2	-	4	7.6	100 ^a	0.240 ± 0.588	2.5	19.0**
Vehicle (DMSO)	+	4	10.0	200	0.010 ± 0.100	3.0	1.0
125 BPA-DA	+	4	8.8	200	0.000 ± 0.000	2.0	0.0
250 BPA-DA	+	4	7.5	200	0.010 ± 0.100	1.0	1.0
350 BPA-DA	+	4	4.8	200	0.015 ± 0.122	5.0	1.5
Positive control (CP) 10	+	4	9.4	100 ^a	0.310 ± 0.775	2.0	21.0**
Vehicle (DMSO)	-	20	8.2	200	0.000 ± 0.000	4.0	0.0
125 BPA-DA	-	20	6.3	200	0.000 ± 0.000	3.5	0.0
250 BPA-DA	-	20	6.2	200	0.010 ± 0.100	2.5	0.1
500 BPA-DA	-	20	4.0	200	0.005 ± 0.071	4.5	0.5
Positive control (MMC) 0.1	-	20	7.1	100 ^a	0.320 ± 0.764	2.0	20.0**

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

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

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

^a Numerical aberrations are out of 100 cells scored.

Reference: Gudi, R. and Rao, M. (2004). Unpublished Report No. AA64EY.331.BTL entitled “*In vitro* Mammalian Chromosome Aberration Test”, dated November 30, 2004, for General Electric Company, Pittsfield, MA, USA; from BioReliance, Rockville, MD, USA.

Reliability: (Klimisch Code 1) Valid without restrictions.

15.0 GENETIC TOXICITY *IN VITRO*

15.1 BACTERIAL TEST

15.1.1

Type:	Bacterial reverse mutation assay (Ames test)
System of testing:	<i>Salmonella typhimurium</i> strains TA98, TA100, TA1535, TA1537, and TA1538
Concentrations:	0, 0.3, 1.0, 3.0, 10 and 30 mg/plate
Metabolic activation:	With []; Without []; With and Without [X]; No data []
Results:	Negative
Cytotoxicity conc.:	With metabolic activation: 30 mg/plate in TA1537 and TA1538 only Without metabolic activation: ≥ 10 mg/plate
Precipitation conc.:	Observed in the cytotoxicity test only at 50 mg/plate
Genotoxic effects:	With metabolic activation: positive []; ambiguous []; Negative [X] Without metabolic activation: positive []; ambiguous []; Negative [X]
Method:	<p><u>Description of test procedure:</u> The <i>Salmonella typhimurium</i> strains were supplied by Dr. Bruce N. Ames, University of California, Berkeley, USA. A preliminary cytotoxicity test was conducted with 10 dose levels of the test substance ranging from 0.01 to 50 mg/plate to determine the doses for the mutagenicity assay. No cytotoxicity was evident; however, the highest dose (50 mg/plate) produced a dense precipitate which precluded the ability to count colonies. For the mutagenicity assay, 100 μL of the appropriate solvent, control or test chemical solution was added to a sterile tube containing 2 mL of top agar and a 100 μL aliquot of the appropriate bacterial culture. Either 0.5 mL of S9 mix or 0.5 mL of phosphate-buffered saline was added for tests with or without metabolic activation, respectively. The top agar mixture was then poured onto a Vogel-Bonner Medium E agar plate. Each dose was tested in triplicate with all five bacterial strains. The plates were transferred to a darkened 37°C incubator after hardening and incubated for 48-72 hours. Bacterial colonies were counted manually or by an automatic counter. An examination of the background lawn was also recorded.</p> <p><u>Solvent and Positive controls:</u> The test substance was suspended in dimethylsulfoxide (DMSO), which also was used as the solvent control. For the nonactivation assay, the following positive control substances were used: 4-nitro-o-phenylenediamine (0.01 mg/plate for strains TA98 and TA1538); sodium azide (0.01 mg/plate for strains TA100 and TA1535); and 9-aminoacridine (0.06 mg/plate for strain 1537). 2-Aminoanthracene (2.5 μg/plate) was used as the positive control substance for the activation assay in all strains.</p> <p><u>Criteria for evaluating results:</u> The spontaneous reversion for the solvent controls should be within the laboratories historical range. The positive controls should demonstrate that the test systems are responsive with</p>

known mutagens. A test chemical is considered to be a bacterial mutagen if the number of revertant colonies is at least twice the solvent control for at least one dose and there is evidence of a dose-related increase in the number of revertant colonies.

Activation system: S9 liver homogenate prepared from Aroclor 1254-induced Sprague-Dawley male rats. The S9 mix was prepared fresh each day of testing.

Year:

1988.

GLP:

Yes [X] No [] ? []

Test substance:

Biphenol Dianhydride (Bisphenol A Dianhydride; BPA-DA; CAS RN 38103-06-9); from General Electric Company; Purity: >99%.

Results:

The number of revertants/plate produced by treatment of the bacteria with the test substance at all concentrations and in all tester strains was reported to be less than or approximately equal to the number of revertants in the vehicle-treated negative control group, with and without metabolic activation. The test material was therefore concluded to not be mutagenic in this assay.

Mean Number of Revertants Per Plate

Activation: None

Dose (mg/plate)	TA98	TA100	TA1535	TA1537	TA1538
Solvent (DMSO)	18	94	19	7	7
0.3	20	72	13	6	9
1.0	17	70	11	2 (t)	3 (t)
3.0	21	77	10	4	6
10	17	55 (s)	6 (s/t)	5	3 (s/t)
30	19	49 (s)	12 (t)	4 (t)	6 (s)
Positive Control	928	1847	1740	208	1130

t = Toxic: Clearing of background lawn, or average number of colonies < ½ solvent control value.

s = Sparse growth of background lawn; counts not included in calculation of mean.

Mean Number of Revertants Per Plate

Activation: Rat Liver S9

Dose (mg/plate)	TA98	TA100	TA1535	TA1537	TA1538
Solvent (DMSO)	30	95	9	6	21
0.3	21	84	11	4	16
1.0	30	82	9	6	15
3.0	25	77	10	5	17
10	19	76	9	1 (s/t)	10 (t)
30	20	77	7	3	10 (t)
Positive Control	2372	1910	83	125	1164

t = Toxic: Clearing of background lawn, or average number of colonies < ½ solvent control value.

s = Sparse growth of background lawn; counts not included in calculation of mean.

Conclusion:	The test substance did not exhibit mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions according to the evaluation criteria.
Reference:	Guzzie, P. J. and Morabit, E. R. (1988). Unpublished report for Project No. 51-511 entitled “Biphenol Dianhydride Salmonella/Microsome (Ames) Bacterial Mutagenicity Assay” dated February 17, 1988 for General Electric Company, Pittsfield, MA, USA; from Bushy Run Research Center, Export, PA, USA.
Reliability:	(Klimisch Code 2) Valid with restrictions. Similar to guideline study; confirmatory assay not performed.

15.1.2

Type:	Bacterial reverse mutation assay
System of testing:	<i>Salmonella typhimurium</i> strains TA98, TA100, TA1535, TA1537, and TA1538
Concentrations:	0, 1, 10, 100, 500, 1000, 2500, 5000 and 10,000 µg/plate
Metabolic activation:	With []; Without []; With and Without [X]; No data []
Results:	Negative
Cytotoxicity conc.:	5000 and 10,000 µg/plate in strains TA1537 and TA1538; and slightly toxic to strains TA1535 and TA100 at 5000 and 10000 µg/plate and to TA98 at 10,000 µg/plate.
Precipitation conc.:	None
Genotoxic effects:	With metabolic activation: positive []; ambiguous []; Negative [X] Without metabolic activation: positive []; ambiguous []; Negative [X]
Method:	Ames et al., Mutation Research 31:347, 1975 <u>Description of test procedure:</u> The plate test consisted of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Approximately 0.1 to 0.2 mL of the test organisms were treated with the test substance in the presence and absence of a metabolic activation system (Aroclor 1254-treated rat liver supernatant). One plate was used per concentration. The plates were incubated for approximately 48 hours at 37 °C, and scored for the number of colonies growing on each plate. <u>Solvent and Positive controls:</u> Dimethylsulfoxide (DMSO) was the solvent for the test substance and served as the solvent control (at 100 µL/plate). For the non-activation assay, the following positive control substances were used: sodium azide (for strains TA1535 and TA100); 2-Nitrofluorene (for strains TA1538 and TA98); and 9-aminoacridine (for strain TA1537). The positive control substance, 2-anthramine, was used for all tester strains in the presence of metabolic activation.

Criteria for evaluating results: The solvent control values must be within the normal historical control range and the presence of a dose response is required for establishing mutagenicity. For strains TA1535, TA1537 and TA1538, a test substance producing a positive response equal to three

times the solvent control value is considered mutagenic. For strains TA98 and TA100, a test substance producing a positive response equal to twice the solvent control value is considered mutagenic. In addition, a positive response must be repeated in a separate assay.

Activation system: S9 liver homogenate prepared from Aroclor 1254-induced Sprague-Dawley male rats. The S9 mix was prepared fresh each day of testing.

Year: 1981

GLP: Yes No ?

Test substance: AR #93480 (Bisphenol A Dianhydride; BPA-DA; CAS RN 38103-06-9); from General Electric Company; Purity: See “Chemical Identity and Use Information” section.

Results: The number of revertants/plate produced by treatment of the bacteria with the test substance at all concentrations and in all tester strains, was reported to be less than or approximately equal to the number of revertants in the solvent-treated negative control group, with and without metabolic activation.

Revertants Per Plate

Activation: None

Dose (µg/plate)	TA1535	TA1537	TA1538	TA98	TA100
Solvent (DMSO)	18	8	10	52	118
Solvent (DMSO)	19	14	17	54	132
1.0	18	12	18	45	99
10	11	6	23	56	128
100	13	7	13	44	123
500	16	8	19	46	111
1000	11	5	17	57	116
2500	14	4	11	56	84
5000	3	0	0	35	43
10,000	1	0	0	4	8
Positive Control	584	190	820	876	1143
Positive Control	645	299	925	913	1249

**Revertants Per Plate
Activation: Rat Liver S9**

Dose (µg/plate)	TA1535	TA1537	TA1538	TA98	TA100
Solvent (DMSO)	15	9	21	68	137
Solvent (DMSO)	17	20	23	73	138
1.0	18	10	28	47	114
10	10	10	30	69	148
100	9	12	18	67	147
500	17	5	20	57	124
1000	18	12	20	65	134
2500	10	9	16	53	104
5000	9	2	4	54	86
10,000	3	0	0	18	42
Positive Control	443	227	1555	1510	1608
Positive Control	450	308	1756	1554	1661

Conclusion: The test substance did not exhibit mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions according to the evaluation criteria.

Reference: Jagannath, D. R. and Brusick, D. J. (1981). Unpublished report for Project No. 20988 entitled “Mutagenicity Evaluation of 02-81-011535-016 AR #93480” dated April 1981 for General Electric, Schenectady, NY, USA; from Litton Bionetics, Inc., Kensington, MD, USA.

Reliability: (Klimisch Code 2) Valid with restrictions. Acceptable study report that meets basic scientific principles.

15.1.3

Type: Bacterial reverse mutation assay (Ames test)
System of testing: *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538; *Saccharomyces cerevisiae* strain D4
Concentrations: 0, 0.1, 1.0, 10, 100 and 500 µg/plate
Metabolic activation: With []; Without []; With and Without [X]; No data []
Results: Negative
Cytotoxicity conc.: With metabolic activation: not stated
Without metabolic activation: not stated
Precipitation conc.: None
Genotoxic effects: With metabolic activation: positive []; ambiguous [];
Negative [X]
Without metabolic activation: positive []; ambiguous [];
Negative [X]
Method: Ames et al., Mutation Research 31:347, 1975
Description of test procedure: The plate test consisted of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Approximately 10⁸ cells were treated with the test substance in the

presence and absence of a metabolic activation system (Aroclor 1254-treated rat liver supernatant). One plate was used per concentration. The plates were incubated for 48 hours at 37 °C, and scored for the number of colonies growing on each plate.

Solvent and Positive controls: Dimethylsulfoxide (DMSO) was the solvent for the test substance and served as the solvent control. For the non-activation assay, the following positive control substances were used: Methylnitrosoguanidine (for strains TA1535, TA100 and D4); 2-Nitrofluorene (for strains TA1538 and TA98); and quinacrine mustard (for strain TA1537). The positive control substances, 2-anthramine (strains TA1535 and TA100), 2-acetylaminofluorene (strains TA1538 and TA98) and 8-aminoquinoline (strain TA1537) were used with metabolic activation. The positive control substance used for DA without activation was not identified in the report.

Criteria for evaluating results: The solvent control values must be within the normal historical control range and the presence of a dose response is required for establishing mutagenicity. For strains TA1535, TA1537 and TA1538, if the solvent control value is within the normal range, a test substance producing a positive response over three concentrations with the lowest increase equal to twice the solvent control is considered mutagenic. For strains TA98, TA100 and D4, a test substance producing a positive response over three concentrations with the lowest increase equal to twice the solvent control (TA100) or two to three times the solvent control (TA98 and D4) is considered mutagenic. In addition, a positive response must be repeated in a separate assay.

Activation system: S9 liver homogenate prepared from Aroclor 1254-induced male Sprague-Dawley rats.

Year: 1977
GLP: Yes [] No [X] ? []
Test substance: 09-77-011154-026 (Bisphenol A Dianhydride; BPA-DA; CAS RN 38103-06-9); from General Electric Company; Purity: See “Chemical Identity and Use Information” section.
Results: The number of revertants/plate produced by treatment of the bacteria with the test substance at all concentrations and in all tester strains was reported to be less than or approximately equal to the number of revertants in the solvent-treated negative control group, with and without metabolic activation.

Revertants Per Plate**Activation: None**

Dose (µg/plate)	TA1535	TA1537	TA1538	TA98	TA100	D4
Solvent (DMSO)	10	14	13	28	169	32
0.1	16	15	11	26	140	38
1.0	19	10	16	31	189	37
10	17	12	16	30	202	41
100	17	15	13	29	206	42
500	12	17	15	28	233	28
Positive Control	653	619	>1000	>1000	>1000	593

Revertants Per Plate**Activation: Rat Liver S9**

Dose (µg/plate)	TA1535	TA1537	TA1538	TA98	TA100	D4
Solvent (DMSO)	17	13	24	35	267	23
0.1	10	18	16	31	196	21
1.0	17	12	24	34	238	10
10	17	14	29	26	217	23
100	11	17	20	38	254	28
500	9	10	13	32	245	25
Positive Control	131	213	574	891	831	48

Conclusion: The test substance did not exhibit mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions according to the evaluation criteria.

Reference: Jagannath, D. R. and Brusick, D. J. (1977). Unpublished report for Project No. 20838 entitled "Mutagenicity evaluation of 09-77-011154-026", dated October 1977 for General Electric, Schenectady, NY, USA; from Litton Bionetics, Inc., Kensington, MD, USA.

Reliability: (Klimisch Code 2) Valid with restrictions. Acceptable study report that meets basic scientific principles.

15.2 NON-BACTERIAL *IN VITRO* TEST (MAMMALIAN CELLS)

Type: *In vitro* mammalian cell gene mutation test (Mouse lymphoma assay)
System of testing: Mouse lymphoma L5178Y cells
Concentration: 0, 50, 75, 100, 125 and 150 µg/mL with activation
0, 25, 50, 75, 100 and 125 µg/mL without activation
Metabolic activation: With []; Without []; With and Without [X];
No data []
Results: Negative with and without metabolic activation.

Cytotoxicity conc.:	With metabolic activation: 500 µg/mL Without metabolic activation: ≥ 150 µg/mL												
Genotoxic effects:	<table border="0"> <tr> <td></td> <td style="text-align: center;">+</td> <td style="text-align: center;">?</td> <td style="text-align: center;">-</td> </tr> <tr> <td>With metabolic activation:</td> <td style="text-align: center;">[]</td> <td style="text-align: center;">[]</td> <td style="text-align: center;">[X]</td> </tr> <tr> <td>Without metabolic activation:</td> <td style="text-align: center;">[]</td> <td style="text-align: center;">[]</td> <td style="text-align: center;">[X]</td> </tr> </table>		+	?	-	With metabolic activation:	[]	[]	[X]	Without metabolic activation:	[]	[]	[X]
	+	?	-										
With metabolic activation:	[]	[]	[X]										
Without metabolic activation:	[]	[]	[X]										
Method:	OECD Test Guideline 476 (1998)												
GLP:	Yes [X] No [] ? []												
Test Substance:	Bisphenol A Dianhydride (BPA-DA; CAS RN 38103-06-9; from General Electric Plastics); Purity: 98.2%.												
Remarks:	<p><u>Description of test procedure:</u> 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 0.05 to 500 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 3x10⁵ cells/mL after 24 hours only. Toxicity was measured as suspension growth of the treated cultures relative to the growth of the solvent control cultures after 48 hours.</p> <p>The mutagenesis assay was carried out by combining 6x10⁶ L5178Y/TK^{+/-} cells, medium or S9 activation mixture and 100 µL dosing solution of test or control article in solvent or solvent alone and incubated for 4 (with and without activation) or 24 (without activation) hours. The positive controls were treated with MMS (at 15 and 20 µg/mL for the 4-hour exposure or 2.5 and 5.0 µg/mL for the 24-hour exposure) and 7,12-DMBA (5.0 and 7.5 µg/mL).</p> <p><u>Expression of the mutant phenotype:</u> For expression of the mutant phenotype, the cultures were counted and adjusted to 3x10⁵ cells/mL at approximately 24 and 48 hours after treatment. Cultures with less than 3x10⁵ cells/mL were not adjusted. For expression of the TK^{-/-} cells, two flasks per culture were cloned for TFT (trifluorothymidine, the selective agent) or VC (viable count). The cells were diluted in cloning medium to concentrations of 3x10⁶ cells/100/mL for the TFT flask and 600 cells/100mL for the VC flask. Cells were plated and incubated for 10-14 days.</p> <p><u>Scoring procedures:</u> The VC plates were counted for the total number of colonies per plate and the total relative growth determined. The TFT-resistant colonies were counted for each culture with ≥ 20% total relative growth (including at least one concentration with ≥ 10% but ≤ 20% total 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.</p>												

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 10^6 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 (2×10^{-4}) then multiplying by 10^6 . 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. 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 10^6 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 10^6 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 10^6 clonable cells over the background level.

Criteria for a Valid Test: For the negative control, the spontaneous mutant frequency of the cultures must be within 20 to 120 TFT-resistant mutants per 10^6 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 10^6 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 BPA-DA cultures, a minimum of four analyzable concentrations with mutant frequency data was required.

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.

Results:

The maximum dose tested in the preliminary toxicity assay was 500 $\mu\text{g/mL}$. Visible precipitate was present at ≥ 150 $\mu\text{g/mL}$ in treatment medium. No visible precipitate was present at concentrations of ≤ 50 $\mu\text{g/mL}$ in treatment medium. The osmolality of the solvent control was 442 mmol/kg and the osmolality of the highest soluble dose, 50 $\mu\text{g/mL}$, was 437 mmol/kg. Suspension growth relative to the solvent controls was 0% at 150 $\mu\text{g/mL}$ without activation with 4- and 24-hour exposures and 7% at 500 $\mu\text{g/mL}$ with S9 activation and 4-hour exposure. Based on the results of this preliminary test the concentrations selected for the mutagenesis assay were 0, 50, 75, 100, 125 and 150 $\mu\text{g/mL}$ with activation and 0, 25, 50, 75, 100 and 125 $\mu\text{g/mL}$ without activation.

Results for cultures treated for four hours (initial assay): Visible precipitate was present at 150 µg/mL. In the non-activated system, suspension growth ranged from 14 to 107%. In the activated system, suspension growth ranged from 103 to 115%. One cloned culture treated with 125 µg/mL without activation exhibited a mutant frequency between 55 and 99 mutants per 10⁶ clonable cells greater than the solvent control. The total growths ranged from 14 to 112% for the non-activated cultures at concentrations of 25 to 125 µg/mL and 108 to 146% for the S9-activated cultures at concentrations of 50 to 150 µg/mL. The results of the initial 4-hour exposure assay were negative in the absence and presence of S9 activation. Because no unique metabolic requirements were known about the test article, only an extended treatment assay was performed in the absence of S9 for a 24-hour exposure period.

Results for cultures treated for 24 hours (extended treatment assay): Visible precipitate was present at 150 µg/mL. Cultures treated with concentrations of 25, 50, 75, 100 and 125 µg/mL were cloned and produced a range in suspension growth of 89 to 115%. One cloned culture (treated with 125 µg/mL) exhibited a mutant frequency of between 55 and 99 mutants per 10⁶ clonable cells over that of the solvent control. A dose-response trend was not observed. The total growths ranged from 98% to 133% at concentrations of 25 to 125 µg/mL. The TFT-resistant colonies for the positive and solvent control cultures from both assays were sized according to diameter over a range from approximately 0.2 to 1.1 mm. The colony sizing for the MMS positive control yielded the expected increase in small colonies, verifying the adequacy of the methods used to detect small colony mutants.

**Cloning Data for L5178Y/TK^{+/-} Mouse Lymphoma Cells Treated with BPA-DA
in the Absence of Exogenous Metabolic Activation
Initial Assay (4-hour exposure)**

Dose Level ($\mu\text{g/mL}$)	Replicate	TFT Colonies				VC Colonies				Mutant Freq. ^a	Induced Mutant Freq. ^b	% Total Growth ^c
		Counts		Mean	Counts		Mean					
0 (solvent)	1	94	90	92	92 \pm 2	176	140	171	162 \pm 16	113	--	--
0 (solvent)	2	34	22	+	28 \pm 5	168	130	127	142 \pm 19	40	--	--
Mean Solvent Mutant Frequency = 76												
25	A	84	67	17	56 \pm 28	165	133	178	159 \pm 19	71	-6	112
25	B	30	37	33	33 \pm 3	151	169	164	161 \pm 8	41	-35	109
50	A	112	103	50	88 \pm 27	176	175	189	180 \pm 6	98	22	110
50	B	38	68	52	53 \pm 12	185	151	186	174 \pm 16	61	-16	106
75	A	83	58	21	54 \pm 25	155	150	162	156 \pm 5	69	-7	18
75	B	65	70	28	54 \pm 19	152	129	131	137 \pm 10	79	3	22
100	A	77	67	73	72 \pm 4	165	153	150	156 \pm 6	93	16	18
100	B	79	76	68	74 \pm 5	151	171	165	162 \pm 8	92	15	15
125	A	63	79	78	73 \pm 7	158	162	164	161 \pm 2	91	14	19
125	B	94	76	86	85 \pm 7	105	122	122	116 \pm 8	147	70	14
Positive Control - Methyl Methanesulfonate ($\mu\text{g/mL}$)												
15	--	104	83	+	94 \pm 9	77	103	93	91 \pm 11	205	129	39
20	--	212	155	129	165 \pm 35	71	48	66	62 \pm 10	536	460	22

Solvent = DMSO

+ = Culture lost

^a Mutant frequency (per 10⁶ surviving cells) = (Average # TFT colonies / average # VC colonies) x 200^b Induced mutant frequency (per 10⁶ surviving cells) = mutant frequency - average mutant frequency of solvent controls^c % Total growth = (% suspension growth x % cloning growth) / 100

**Cloning Data for L5178Y/TK^{+/+} Mouse Lymphoma Cells Treated with BPA-DA
in the Presence of Exogenous Metabolic Activation
Initial Assay (4-hour exposure)**

Dose Level ($\mu\text{g/mL}$)	Replicate	TFT Colonies				VC Colonies				Mutant Freq. ^a	Induced Mutant Freq. ^b	% Total Growth ^c
		Counts		Mean	Counts		Mean					
0 (solvent)	1	59	68	35	54 \pm 14	212	101	65	126 \pm 63	86	--	--
0 (solvent)	2	80	17	89	62 \pm 32	80	212	232	175 \pm 67	71	--	--
Mean Solvent Mutant Frequency = 78												
50	A	73	63	55	64 \pm 7	169	152	199	173 \pm 19	73	-5	130
50	B	50	13	64	42 \pm 22	181	194	191	189 \pm 6	45	-33	141
75	A	71	60	81	71 \pm 9	146	168	162	159 \pm 9	89	11	121
75	B	72	86	84	81 \pm 6	195	159	166	173 \pm 16	93	15	130
100	A	96	85	93	91 \pm 5	215	198	158	190 \pm 24	96	18	146
100	B	94	78	81	84 \pm 7	195	190	156	180 \pm 17	94	15	138
125	A	101	78	70	83 \pm 13	177	196	202	192 \pm 11	87	8	133
125	B	101	87	90	93 \pm 6	162	177	96	145 \pm 35	128	49	108
150*	A	89	94	79	87 \pm 6	177	194	179	183 \pm 8	95	17	125
150*	B	85	80	57	74 \pm 12	178	157	182	172 \pm 11	86	8	122
Positive Control - 7,12 Dimethylbenz(a)anthracene ($\mu\text{g/mL}$)												
2.5	--	198	51	60	103 \pm 67	54	50	80	61 \pm 13	336	258	35
4	--	238	269	275	261 \pm 16	99	59	50	69 \pm 21	752	674	14

Solvent = DMSO

* Precipitating concentration

^a Mutant frequency (per 10⁶ surviving cells) = (Average # TFT colonies / average # VC colonies) x 200^b Induced mutant frequency (per 10⁶ surviving cells) = mutant frequency - average mutant frequency of solvent controls^c % Total growth = (% suspension growth x % cloning growth) / 100

**Cloning Data for L5178Y/TK^{+/+} Mouse Lymphoma Cells Treated with BPA-DA
in the Absence of Exogenous Metabolic Activation
Extended Treatment Assay (24-hour exposure)**

Dose Level (µg/mL)	Replicate	TFT Colonies				VC Colonies				Mutant Freq. ^a	Induced Mutant Freq. ^b	% Total Growth ^c
		Counts		Mean	Counts		Mean					
0 (solvent)	1	60	32	57	50 ± 13	158	160	143	154 ± 8	65	--	--
0 (solvent)	2	24	30	37	30 ± 5	120	131	127	126 ± 5	48	--	--
Mean Solvent Mutant Frequency = 56												
25	A	72	32	39	48 ± 17	171	140	160	157 ± 13	61	4	100
25	B	39	40	40	40 ± 0	158	146	137	147 ± 9	54	-2	98
50	A	47	54	24	42 ± 13	175	165	162	167 ± 6	50	-7	113
50	B	117	86	40	81 ± 32	131	160	155	149 ± 13	109	53	108
75	A	40	45	33	39 ± 5	183	130	143	152 ± 23	52	-5	114
75	B	32	24	32	29 ± 4	136	164	198	166 ± 25	35	-21	133
100	A	104	109	46	86 ± 29	190	145	142	159 ± 22	109	52	111
100	B	66	78	78	74 ± 6	137	163	197	166 ± 25	89	33	127
125	A	87	41	94	74 ± 24	176	169	117	154 ± 26	96	40	113
125	B	72	74	86	77 ± 6	122	119	168	136 ± 22	113	57	113
Positive Control - Methyl Methanesulfonate (µg/mL)												
5	--	163	136	132	144 ± 14	66	76	86	76 ± 8	378	322	40
7.5	--	214	156	140	170 ± 32	70	72	85	76 ± 7	449	393	32

Solvent = DMSO

^a Mutant frequency (per 10⁶ surviving cells) = (Average # TFT colonies / average # VC colonies) x 200

^b Induced mutant frequency (per 10⁶ surviving cells) = mutant frequency - average mutant frequency of solvent controls

^c % Total growth = (% suspension growth x % cloning growth) / 100

Conclusions: All criteria for a valid study were met as described in the protocol. The results of the L5178Y/TK^{+/+} Mouse Lymphoma Mutagenesis Assay indicate that, under the conditions of this study, the mutagenicity of BPA-DA was concluded to be negative without activation with 4- and 24-hour exposures and negative with S9 activation with a 4-hour exposure.

Reference: San, R.H.C. and Clarke, J.J. (2004). Unpublished Report No. AA64EY.704.BTL entitled “*In vitro* mammalian cell gene mutation test (L5178Y/TK^{+/+} mouse lymphoma assay)”, November 22, 2004 for General Electric Company, Pittsfield, MA, USA; from BioReliance Corp., Rockville, MD, USA.

Reliability: (Klimisch Code 1) Valid without restrictions.

16.0 REPEATED DOSE TOXICITY

Species/Strain: Rat/Sprague-Dawley Crl:COBS®, CD®, (SD) Br
Sex: Female []; Male []; Male/Female [X]; No data []
Route of Administration: Oral, Dietary feed
Exposure Period: 30 days
Frequency of Treatment: Daily
Post Exposure
Observation Period: None
Dose: 0, 1, 2, and 4% of BPA-DA in basal diet (approximately 646 – 765, 1277 – 1490, and 2750 – 3160 mg/kg/day, respectively)
Control Group: Yes [X] No [] No data []
Concurrent no treatment [X] Concurrent vehicle [] Historical []
NOAEL: 4% (approximately 2750 to 3160 mg/kg/day)
LOAEL: > 4%
Method: Not stated.
Year: 1982
GLP: Yes [X] No [] ? []
Test Substance: Bisphenol A Dianhydride (BPA-DA; CAS RN 38103-06-9); Lot UI-82-4 from General Electric Company; Purity: See “Chemical Identity and Use Information” section.

Remark: Test procedure: Groups of 10 rats/sex were fed BPA-DA at concentrations of 0, 1, 2 and 4% in the diet. All rats were observed for mortality twice each day. Clinical signs and body weights were recorded at initiation and weekly thereafter. Food consumption was recorded weekly. After 31 days of treatment, all surviving rats were weighed, killed and a gross necropsy was performed. At necropsy, the liver and kidneys of each animal were weighed and organ to body weight ratios determined. The following tissues were preserved from all animals: brain, pituitary, thoracic spinal cord, eyes, salivary glands, thyroid, parathyroids, thymus, trachea, esophagus, lung, heart, liver, spleen, kidneys, adrenals, stomach, pancreas, duodenum, jejunum, ileum, colon, cecum, mesenteric lymph node, urinary bladder, testes with epididymides and prostate (males), ovaries and uterus (females), femur, costal bone marrow, skeletal muscle, and all gross lesions. Microscopic evaluation was conducted on sections of the lungs, liver, brain and kidneys from rats of all treatment groups. Reproductive organs were not evaluated histologically.

The following statistical tests were utilized to evaluate body weight changes, total food consumption and organ weights: Bartlett’s test for homogeneity of variance and one-way classification analysis of variance (ANOVA). Since the ANOVA proved to be not significant for all of the analyses, no other tests were performed. All analyses were performed at the 5% one-tailed probability level.

Results: No deaths occurred during the study. No compound-related clinical observations were noted throughout the study. Body weight and food consumption data of the compound-treated males and females were

generally comparable to those of their respective controls. Individual and mean terminal body weights, absolute organ weights and organ weights relative to terminal body weight were not affected by treatment. No compound-related organ or tissue changes were evident macroscopically or microscopically.

- Reference: Burdock, G. A. and Kundzins, W. (1982). Unpublished Report No. 349 262 entitled “Thirty-Day Subchronic Oral Toxicity Study in Rats, BPA-BI and BPA-DA”, dated December 17, 1982 for General Electric Company, Mount Vernon Indiana, USA; from Hazleton Laboratories America, Inc., Vienna, VA, USA; and
Burdock, G. A. (1984). Unpublished addendum to Final Report No. 349 262 entitled “Thirty-Day Subchronic Oral Toxicity Study in Rats, BPA-BI and BPA-DA”, dated December 22, 1984 for General Electric Company, Mount Vernon Indiana, USA; from Hazleton Laboratories America, Inc., Vienna, VA, USA.
- Reliability: (Klimisch Code 2) Valid with restrictions. Minimal data collected for a repeat dose study.

17.0 REPRODUCTIVE TOXICITY

Type: Fertility [] One generation study [] Two generation study []
Other [X]

Species/Strain: Rat/CD[®] (Sprague-Dawley)

Sex: Female [] Male [] Male/Female [X] No data []

Route of Administration: Oral, gavage

Exposure Period: Males = 4 weeks (2 week pre-breeding, 2 weeks mating)
Females = ~7 weeks (2 weeks prebreed, 2 weeks mating, 3 weeks gestation, and lactation through postnatal day 4)

Frequency of Treatment: Daily

Premating Exposure Period: 2 weeks

Duration of Test: F0 generation males 4 weeks
F0 generation females postnatal day 4

Dose: 0, 100, and 1000 mg/kg/day

Control Group: Yes [X]; No []; No data []
Concurrent no treatment [] Concurrent vehicle [X] Historical []

NOAEL Parental: Male: 100 mg/kg/day
Female: 100 mg/kg/day

NOAEL Reproduction: >1000 mg/kg/day (males and females)

NOAEL F1 Offspring: >1000 mg/kg/day (males and females)

Method: OECD Test Guideline 421 (1995); due to the limited toxicity observed in previous studies for BPA-BI, only two BPA-DA dose groups were used

GLP: Yes [X] No [] ? []

Test Substance: Bisphenol A Dianhydride (BPA-DA; CAS RN 38103-06-9); Lot U10054 from General Electric Company; Purity: 98.2%

Remark: Test procedure: Male and female CD[®] (Sprague-Dawley) rats (the F0 generation) were administered BPA-DA orally by gavage at 0, 100, and 1000 mg/kg/day at a dose volume of 5 mL/kg/day in corn oil, ten/animals/sex/dose, for two weeks of prebreed exposure and two weeks of mating for F0 male and female parental animals. F0 females continued to be dosed for three weeks of gestation and through postnatal day (pnd) 3. Body weights for the F0 males and females were recorded weekly during the prebreed and mating periods for both sexes and for F0 females during gestation. During lactation, F0 female body weights were obtained on pnd 0 and 4. Feed consumption was recorded weekly for the F0 males and females during the prebreed period, but not during the mating period. Feed consumption was recorded for the F0 females during gestation and through pnd 4 of lactation. 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. F0 males were sacrificed following the breeding period (after 28 days of dosing). F0 females with litters were sacrificed on pnd 4 and F0 females that did not produce a litter were sacrificed on gestation day (gd) 26 or 26 days after mating.

On the day of birth (pnd 0), all live F1 pups were counted, sexed, weighed and examined as soon as possible. All stillborn pups or pups that died on the date of birth were sexed and counted. All pups were examined daily from birth through pnd 4 for survival and physical abnormalities. Any pups dying during lactation were necropsied, if possible. On pnd 4, all live pups were examined sexed and weighted, then euthanized and discarded without further evaluation.

At the F0 parental animal necropsy, the following tissues were weighed and retained: testes, epididymides, prostate, seminal vesicles, ovaries, uterus. All gross lesions were also retained. Histopathology was performed on all retained reproductive tissues for the high dose and control males and females with special emphasis on stages of spermatogenesis in the male gonads and histopathology of interstitial testicular cell structure (10/sex/group). The uteri from the F0 females that failed to produce a litter by gd 26 or by 26 days post-mating were stained with potassium ferricyanide for confirmation of pregnancy.

Results

The following is a discussion of the F0 adult systemic toxicity, F0 parental reproductive toxicity, and F1 offspring toxicity. Summary data tables are also provided. The discussion focuses on treatment-related effects. Other changes noted in the tables were considered random, due to biological variation, and not related to treatment.

F0 Adult Systemic Toxicity: A table follows the results write-up that provides a summary of F0 adult systemic toxicity.

Males: There were no treatment-related deaths for the F0 males. For parental males at 1000 mg/kg/day, body weight was reduced on study days (sd) 21 and 28, by 7% and 5% respectively. Body weight change was reduced at 1000 mg/kg/day for sd 14 to 21 and 0 to 28. At 1000 mg/kg/day, feed consumption (g/day) was increased from sd 7 to 14 and feed consumption (g/kg/day) was increased throughout the entire prebreed period. At 100 mg/kg/day, there were no effects on body weight, body weight changes or feed consumption. At 1000 mg/kg/day, treatment-related clinical observations were recorded for five males with audible respiration, two males with gasping, four males with sneezing, and one male with chromodacryorrhea. Other findings were not considered related to treatment with the exception of post-dose rooting. Post-dose rooting was defined as the animal digging or moving its bedding with its snout post-dosing, possibly to mitigate the adverse taste. Post-dose rooting is considered to be a behavioral response to taste aversion to the dosing formulations and not a toxic sign. Since there was a dose-related increase in the incidence of post-dose rooting (1, 2 and 5 males in the 0, 100 and 1000 mg/kg/day groups, respectively), it was presumed that the increasing concentrations of BPA-DA across groups caused the adverse taste reaction. At necropsy the mean final body weight was reduced by 5% at 1000 mg/kg/day compared to controls, but the absolute and relative organ weights were equivalent across all groups. There were no treatment-related macroscopic or microscopic findings.

Females: There were no treatment-related deaths for the F0 females. There were no significant changes in F0 female body weights or feed consumption during the prebreed and mating periods; however, there was a decrease in body weight change from sd 7-14 at 1000 mg/kg/day. During gestation, there were no significant changes in body weight or body weight change for the F0 females; however, feed consumption (g/kg/day) was increased from gd 7 to 14 at 100 and 1000 mg/kg/day compared to controls. During lactation, there were no significant differences between groups in F0 maternal body weights, body weight change or feed consumption values. Treatment-related clinical observations at 1000 mg/kg/day included two to three females with audible respiration, sneezing and/or chromodacryorrhea. Other findings were not considered related to treatment except for an increase in the incidence of post-dose rooting. Post-dose rooting is considered to be a behavioral response to taste aversion to the dosing formulations and not a toxic sign. Since there was a dose-related increase in the incidence of post-dose rooting (0, 2 and 3 females in the 0, 100 and 1000 mg/kg/day groups, respectively), it was presumed that the increasing concentrations of BPA-DA across groups caused the adverse taste reaction. At necropsy, mean final body weights of the F0 females were equivalent across all dose groups. The absolute weight and weight relative to final body weight of the paired ovaries were significantly reduced at 1000 mg/kg/day. There were no treatment-related macroscopic or microscopic findings.

 Summary of F0 Adult Systemic Toxicity – Key Parameters and Statistically Significant Differences

Bisphenol A Dianhydride (mg/kg/day)	0	100	1000
<u>F0 MALES</u>			
Deaths	0/10	0/10	0/10
Body Weights			
sd 21	---	---	↓
sd 28	---	---	↓
Weight Change			
sd 14-21	---	---	↓
sd 0-28	---	---	↓
Feed Consumption:			
g/day			
sd 7-14	---	---	↓↓
g/kg/day			
sd 0-7	---	---	↓↓
sd 7-14	---	---	↓↓↓
sd 0-14	---	---	↓↓↓
<u>Necropsy</u>			
Final Body Weight at Necropsy	---	---	↓
Organ Weights	---	---	---
<u>F0 FEMALES</u>			
Deaths	0/10	0/10	0/10
<u>Prebreed, Mating, and Postmating (sd 0-42) Exposure</u>			
Body Weights	---	---	---
Weight Change			
sd 7-14	---	---	↓
Feed Consumption:			
g/day	---	---	---
g/kg/day	---	---	---
<u>Gestation</u>			
Body Weights	---	---	---
Weight Change	---	---	---
Feed Consumption:			
g/day	---	---	---
g/kg/day			
gd 7-14	---	↑↑	↑
<u>Lactation (pnd 0-21)</u>			
Body Weights	---	---	---
Weight Change	---	---	---
Feed Consumption:			
g/day	---	---	---
g/kg/day	---	---	---
<u>Necropsy</u>			
Final Body Weight at Necropsy	---	---	---
Organ Weights			
Paired Ovary Weight	---	---	↓↓
Relative Paired Ovary Weight	---	---	↓

↑, ↑↑, ↑↑↑ = statistically significant increase; p<0.05, p<0.01 and p<0.001, respectively

↓, ↓↓, ↓↓↓ = statistically significant decrease; p<0.05, p<0.01 and p<0.001, respectively

--- = no statistically significant difference

Results (Continued): F0 Parental Reproductive Toxicity: The following Table presents a summary of F0 parental reproductive toxicity. During the post mating period, there were 9, 9, and 8 females in the 0, 100, and 1000 mg/kg/day dose groups, respectively, that were determined to be sperm positive; however, the total number of females confirmed pregnant at study completion was 9, 10 and 9, respectively. One pregnant female in the 100 mg/kg/day group was not identified as being sperm positive; one female each in the control and 1000 mg/kg/day groups was not pregnant, and one pregnant female at 1000 mg/kg/day did not deliver a litter. There was a statistically significant increase in precoital interval at 1000 /mg/kg/day although the increase was only approximately one day longer than the controls. There were no significant effects of exposure to BPA-DA on F0 fertility, mating, pregnancy, preimplantation or postimplantation loss per litter, or the number of dead pups at birth.

Summary of F0 Parental Male and Female Reproductive Toxicity

Bisphenol A Dianhydride (mg/kg/day)	F0		
	0	100	1000
F0 Females			
No. Females on Study	10	10	10
No. Females Paired	10	10	10
No. Females that Mated	10	10	9
Mating Index (# females mated/# females paired)	100.0	100.0	90.0
No. Pregnant Females	9	10	9
Fertility Index (# pregnant females/# females that mated)	90.0	100.0	100.0
No. of Females with Live Litters (pnd 0)	9	10	8 ^a
Gestational Index (# females with live litters/# females pregnant)	100.0	100.0	88.9
F0 Males			
No. Males on Study	10	10	10
No. Males Paired	10	10	10
No. Males that Mated	10	10	9
Mating Index (# males mated/# males paired)	100.0	100.0	90.0
No. Males Siring Litters	9	10	9
Fertility Index (# males siring litters/# males that mated)	90.0	100.0	100.0
Pregnancy Index (# females with live litters/# males that mated)	90.0	100.0	88.9
Precoital Interval (days)	1.8 ± 0.2	2.1 ± 0.4	3.1 ± 0.5*
Gestational Length (days)	22.0 ± 0.0	22.3 ± 0.2	22.3 ± 0.2

--Continued--

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Summary of F0 Parental Male and Female Reproductive Toxicity (continued)

Bisphenol A Dianhydride (mg/kg/day)	F0		
	0	100	1000
No. Live Litters			
Postnatal Day 0	9	10	8
Postnatal Day 4	9	10	8
No. Corpora Lutea per Dam	15.33 ± 1.25	14.30 ± 1.32	14.10 ± 1.51
% Preimplantation Loss per Litter	5.20 ± 2.45	8.69 ± 3.88	6.33 ± 2.91 ^b
Average No. Implantation Sites per Litter	15.89 ± 1.12	14.20 ± 1.55	16.00 ± 1.00
% Postimplantation Loss per Litter	5.77 ± 1.69	14.70 ± 5.52	19.82 ± 10.37
Average No. of Live Pups on Postnatal Day 0	14.8 ± 1.2	12.7 ± 1.7	13.4 ± 1.1
Average No. of Dead Pups on Postnatal Day 0	0.2 ± 0.1	0.0 ± 0.0	0.6 ± 0.3
Average Total Number of Pups on Postnatal Day 0	15.0 ± 1.1	12.7 ± 1.7	14.0 ± 1.0
Stillbirth Index (# dead on pnd 0/total # on pnd 0)	1.9 ± 1.3	0.0 ± 0.0	5.0 ± 2.9
Live Birth Index (# live on pnd 0/total# on pnd 0)	98.1 ± 1.3	100.0 ± 0.0	95.0 ± 2.9
4 Day Survival Index (# surviving 4 days/# live on pnd 0)	98.8 ± 0.8	92.8 ± 4.9	98.2 ± 1.8

^a A female was pregnant (20 implantation sites at necropsy) but did not deliver a litter.

^b One female had corpora lutea, but no implantation sites; therefore, preimplantation loss could not be calculated.

Results (Continued): F1 Offspring Toxicity: The following Table presents a summary of F1 offspring toxicity parameters. There was no evidence of F1 offspring toxicity at any dose.

Summary of F1 Offspring Toxicity

Bisphenol A Dianhydride (mg/kg/day)	F1		
	0	10	100
No. Live Litters			
Postnatal Day 0	9	10	8
Postnatal Day 4	9	10	8
Average No. of Live Pups per Litter (pnd 0)	14.8 ± 1.2	12.7 ± 1.7	13.4 ± 1.1
Average No. of Live Pups per Litter (pnd 4)	14.6 ± 1.1	12.3 ± 1.7	13.3 ± 1.2
Average Pup Body Weight (g) per Litter (pnd 0)	6.33 ± 0.14	7.03 ± 0.17**	6.40 ± 0.14
Average Male Body Weight (g) per Litter (pnd 0)	6.45 ± 0.17	7.16 ± 0.19*	6.49 ± 0.16
Average Female Body Weight (g) per Litter (pnd 0)	6.22 ± 0.13	6.91 ± 0.17**	6.30 ± 0.13
Average Pup Body Weight (g) per Litter (pnd 4)	9.96 ± 0.33	11.22 ± 0.52	10.81 ± 0.51
Average Male Body Weight (g) per Litter (pnd 4)	10.20 ± 0.36	11.44 ± 0.58	11.02 ± 0.52
Average Female Body Weight (g) per Litter (pnd 4)	9.71 ± 0.30	10.93 ± 0.54	10.63 ± 0.52
% Percent Male Pups per Litter (pnd 0)	54.9 ± 5.1	48.5 ± 3.5	44.4 ± 3.9
% Percent Male Pups per Litter (pnd 4)	55.0 ± 5.2	60.2 ± 6.5	48.8 ± 2.7

* p < 0.05; ** p < 0.01

Conclusion:	Minimal systemic toxicity was present in males and females through the course of the study at 1000 mg/kg/day. In the F0 males, the only adverse effects were respiratory signs (considered to be related to the irritant properties of BPA-DA), a 5 to 7 % reduction in body weights, and reductions in body weight changes. In the F0 females, the only adverse effects were decreases in high dose body weight change (sd 7 to 14) and treatment related clinical signs at the high dose. At the high dose there was an increase in the precoital interval, but no effect on fertility. There was no evidence of reproductive toxicity in the F0 females at any dose, or any toxicity in the F1 offspring. At necropsy, for the F0 males and females, there were no treatment effects with the exception of decreases in the absolute and relative paired ovary weights. Based on these results, the NOAEL for the F0 male and female systemic toxicity was 100 mg/kg/day. The NOAEL for F0 reproductive toxicity was >1000 mg/kg/day for both sexes. The NOAEL for F1 offspring toxicity was >1000 mg/kg/day.
Reference:	Tyl, R.W., Myers, C.B. and Marr, M.C. (2005). Unpublished Report No. 08627.005.200 entitled “Reproductive/Developmental Toxicity Screening Test of Bisphenol A Dianhydride (BPA-DA; CAS No. 38103-06-9) Administered via Oral Gavage to CD® (Sprague-Dawley) Rats (OECD 421)”, dated July 14, 2005 for General Electric Company, Pittsfield, MA from RTI International, Research Triangle Park, NC, USA.
Reliability:	(Klimisch Code 1) Valid without restrictions.

18.0 DEVELOPMENTAL TOXICITY/TERATOGENICITY

18.1

Species/Strain:	Rabbit, New Zealand White
Sex:	Female <input checked="" type="checkbox"/> ; Male <input type="checkbox"/> ; Male/Female <input type="checkbox"/> ; No data <input type="checkbox"/>
Route of Administration:	Oral (gavage)
Duration of Test:	29 days
Exposure Period:	Days 6 through 18 of gestation
Frequency of Treatment:	Daily
Dose:	1000 mg/kg/day
Control group:	Yes <input checked="" type="checkbox"/> No <input type="checkbox"/> No data <input type="checkbox"/> Concurrent no treatment <input type="checkbox"/> Concurrent vehicle <input checked="" type="checkbox"/> Historical <input type="checkbox"/> Positive Control (thalidomide)
NOEL Maternal Toxicity:	Not established (based on decrease in body weight gain)
NOEL Teratogenicity:	> 1000 mg/kg/day
GLP:	Yes <input checked="" type="checkbox"/> No <input type="checkbox"/> ? <input type="checkbox"/>
Test Substance:	Bisphenol A Dianhydride (BPA-DA; CAS RN 38103-06-9); Lot UI-82-4 from General Electric Company; Purity: See “Chemical Identity and Use Information” section.
Method:	Not stated.

Remark:

Test procedure: Ninety mature New Zealand White female rabbits were obtained from Dutchland Laboratory Animals, Inc., Denver, PA for use in this study. The animals were acclimated for a minimum of 22 days prior to the initiation of the study. During the period of acclimation, the rabbits were examined for general health and appearance. The animals were uniquely identified by ear tag and provided commercial rabbit ration (Purina Lab Rabbit Chow®) and tap water *ad libitum*. The environment of the study room was maintained at 70-78 °C, relative humidity of 53-86% and a 12-hour light/dark cycle. At Day 0 of gestation, the body weights ranged from 2845 to 4700 grams. The animals were artificially inseminated with sperm from the laboratory breeding stock five hours after induction of ovulation with chorionic gonadotropin. Five groups were included in this study; for the purposes of this summary, only three groups (control, positive control, and BPA-DA treated) will be discussed. Sixteen animals per group (to obtain at least 12 pregnant) were treated with vehicle (0.5% carboxymethyl cellulose), positive control (thalidomide; 150 mg/kg/day) or BPA-DA (1000 mg/kg/day). Thalidomide and BPA-DA were suspended in vehicle to provide dose volumes of 1.5 and 2.5 mL/kg, respectively. Control dose volume was 4.0 mL/kg. The dose was administered from gestation day (gd) 6 through 18, approximately the same time each day, and was based on each individual body weight on gd 6 (starting on gd 11, two animals in the control group, four animals in the thalidomide group and three animals in the BPA-DA group were dosed based on gd 11 body weight). All of the animals were observed daily for mortality, moribundity and clinical signs. Body weights were recorded on gd 0, 6, 11, 15, 19 and 29. Individual food consumption was recorded weekly. On gd 29, the animals were sacrificed, examined for gross pathology of the external surface and viscera, and the uterus excised and weighed. The fetuses were taken by cesarean section and the following recorded for each litter: the number of corpora lutea per ovary; the number and placement of uterine implantation sites; live and dead fetuses; early and late resorptions; and any other abnormalities. Fetuses were removed from the placenta, individually identified, examined externally, weighed and measured from the frontal-parietal suture to the base of the tail (crown-rump distance). Cesarean sections were also performed on dams that were found dead, sacrificed moribund or sacrificed due to early delivery. The number of corpora lutea, implantations, resorptions and live or dead fetuses was recorded.

Visceral Examination of Fetuses: The unfixed fetuses underwent visceral examination according to the method of Staples. All of the fetuses were opened by longitudinal incision, the sex determined and examined grossly both externally and internally. Major organs were inspected *in situ* with special attention to the heart and major blood vessels. The heads of approximately one-third of the fetuses were removed, fixed in Bouin's solution, sectioned by Wilson's freehand sectioning technique for examination of the eyes, palate, nasal septum and brain. The prepared sections were then re-examined against a light box with the aid of magnification.

Skeletal Examination of Fetuses: Following visceral examination, all fetuses (minus the head for approximately one-third of the fetuses) were eviscerated and placed in 95% ethyl alcohol. After fixation and dehydration, the skeletons were stained in a potassium hydroxide-alizarin red solution. The skull, vertebral column, rib cage, pectoral and pelvic girdles, long bones and extremities of each skeleton were examined for degree of ossification, bone alignment, and possible anomalies. Examinations were performed with the aid of magnification on a light box.

Statistical Analyses: Mean maternal body weight changes, food consumption, percentage data (implantations, resorptions and males), and fetal viability were analyzed in the following order: Levene's test for homogeneity of variance; if the variances proved to be homogeneous, the data were analyzed by one-way classification analysis of variance (ANOVA); if the variance proved to be heterogeneous, a series of transformations was performed until homogeneity was achieved followed by ANOVA. If ANOVA was significant, the Games and Howell modification of the Tukey-Kramer honestly significant difference test was used to compare groups. Pregnancy rates were analyzed by Fisher's exact test. External, visceral, and skeletal anomalies were evaluated by a multiple proportions test. Analysis of covariance (ANCOVA) was used to analyze mean fetal weights and lengths with the litter used as the experimental unit. Levene's test and ANOVA were evaluated at the 5% one-tailed probability level. Control vs. treatment group mean comparisons were evaluated at the 5% two-tailed probability level.

Range-finding study: A range-finding study was conducted to select the dose used in this study. Four non-pregnant New Zealand White rabbits were dosed with BPA-DA at 2000 mg/kg/day for the first six days and, following a three-day rest period, the dose was changed to 1000 mg/kg/day for the remaining seven days of the study. Compound-related clinical signs included depression, slight depression, anorexia, soft feces, ataxia and compound-colored urine stains in the pan. Two of the four rabbits died; one on Day 12 and the other on Day 16. Based on this study, 1000 mg/kg/day was selected for the teratology study.

Results:

Weight loss was observed in the thalidomide- and BPA-DA-treated groups during the treatment period. Statistical evaluation of body weight change did not, however, reveal any significant differences between treated and control groups. No effects on food consumption or gross pathology of the dams were observed. The following tables summarize the fetal results:

Summary of Mean Ovarian, Uterine, and Litter Data

Parameter	Control (Vehicle)	Thalidomide (Positive Control)	BPA-DA (1000 mg/kg/day)
Number of dams	16	16	16
Number pregnant	14	16	13

Pregnancy rate (%)	88	100	81
Number dams surviving to gd 29 (survival rate)	13 (93%)	15* (100%)	12 (92%)
Mean number of Corpora lutea	13.4	12.2	11.4
Implantations	9.4	8.3	6.5
Resorptions-total	1.2	5.3	2.1
Fetuses – live	7.5	3.4	4.5
– dead	0.5	0	0.1
Indices (mean per litter)			
Implantation efficiency (%)	73.6	68.1	57.0
Incidence of resorption (%)	17.2	61.0	31.0
Incidence of fetal mortality (%)	3.8	0	0.9
Incidence of fetal viability (%)	79.2	39.1	68.1
Live fetuses			
Mean body weight (g) – males	40.91	38.36	42.80
– females	39.90	37.60	41.78
Mean length (cm) – males	9.49	9.03	9.42
– females	9.33	8.92	9.39
Percent Males	51.5	58.0	47.1
Mean uterine weights – gravid (g)	485.3	228.3	315.4

* One animal died accidentally on gd 8

Summary of Mean Incidence of Abnormal Fetuses per Litter

Parameter	Control (Vehicle)	Thalidomide (Positive Control)	BPA-DA (1000 mg/kg/day)
External			
# of litters examined	12	11	9
# of litters with anomalous fetuses	2	10*	3
% of litters with anomalous fetuses	16.7	90.9	33.3
Mean values (per litter)			
# of fetuses with variants	0	0.5	0
Incidence of variants (%)	0	14.4	0
# of fetuses with anomalies	0.3	2.7	0.6
Incidence of anomalies (%)	2.4	64.1	8.2
Visceral – Fetal Heads			
# of litters examined	12	9	8
# of litters with anomalous fetuses	0	3	0
% of litters with anomalous fetuses	0	33.3	0
Mean values (per litter)			
# of fetuses with variants	0	0.1	0
Incidence of variants (%)	0	3.7	0
# of fetuses with anomalies	0	0.4	0
Incidence of anomalies (%)	0	16.7	0
Visceral – Torso and Limbs			
# of litters examined	12	11	9
# of litters with anomalous fetuses	0	8*	1
% of litters with anomalous fetuses	0	72.7	11.1

Summary of Mean Incidence of Abnormal Fetuses per Litter

Parameter	Control (Vehicle)	Thalidomide (Positive Control)	BPA-DA (1000 mg/kg/day)
Mean values (per litter)			
# of fetuses with variants	0.8	2.9	0.4
Incidence of variants (%)	11.1	63.9	8.7
# of fetuses with anomalies	0	1.5	0.1
Incidence of anomalies (%)	0	38.8	1.6
Skeletal – Skulls			
# of litters examined	12	11	9
# of litters with anomalous fetuses	0	2	0
% of litters with anomalous fetuses	0	18.2	0
Mean values (per litter)			
# of fetuses with variants	0.5	1.6	1.2
Incidence of variants (%)	11.3	60.9	33.7
# of fetuses with anomalies	0	0.2	0
Incidence of anomalies (%)	0	11.4	0
Skeletal – Torso and Limbs			
# of litters examined	12	11	9
# of litters with anomalous fetuses	0	10*	1
% of litters with anomalous fetuses	0	90.9	11.1
Mean values (per litter)			
# of fetuses with variants	0.6	3.9	0.8
Incidence of variants (%)	6.9	91.7	16.4
# of fetuses with anomalies	0	2.2	0.1
Incidence of anomalies (%)	0	54.4	1.2

* Statistically significantly different from vehicle control group ($p < 0.05$)

Conclusion: There were no differences from control in the thalidomide or BPA-DA dose groups for maternal, ovarian or uterine data. The thalidomide-treated group exhibited changes consistent with the known teratogenic effect of this compound. The thalidomide group may additionally have had an increase in resorptions and exhibited a possible fetotoxic effect as demonstrated by slightly decreased mean body weights and lengths of the fetuses. There were no effects on any fetal parameters from BPA-DA treatment. Based on the results of this study, BPA-DA is not a developmental toxin.

Reference: Burdock, G. A. (1983). Unpublished Report No. 349-267 entitled “Teratogenicity Study in Rabbits, PI, BPA-BI, BPA-DA”, dated August 25, 1983 for General Electric Company, Pittsfield, MA, USA; from Hazleton Laboratories America, Inc., Vienna, VA, USA.

Reliability: (Klimisch Code 1) Reliable without restrictions.

18.2

Species/Strain: Rat; CrI:CD[®](SD)BR

Sex: Female [X]; Male []; Male/Female []; No data []

Route of Administration: Oral (gavage)

Duration of Test: 20 days

Exposure Period: Days 6 through 15 of gestation
Frequency of Treatment: Daily
Dose: 1000 mg/kg/day
Control group: Yes No No data
Concurrent no treatment Concurrent vehicle Historical

NOEL Maternal
Toxicity: None – based on decrease maternal weight gain
NOEL Teratogenicity: > 1000 mg/kg/day
GLP: Yes No ?

Test Substance: Bisphenol A Dianhydride (BPA-DA; CAS RN 38103-06-9); from General Electric Company; Purity: See “Chemical Identity and Use Information” section.

Method: Not stated.

Remark: Test procedure: One hundred twenty, successfully mated Sprague-Dawley female rats, obtained from Charles River Breeding Laboratories, Inc., (Portage, MI), were used in this study. Prior to in-house breeding, the rats were examined for general health and appearance. The animals were uniquely identified by ear tag and provided commercial rat ration (Purina Certified Rodent Chow®) and tap water *ad libitum*. The environment of the study room was monitored daily and a 12-hour light/dark cycle was used. Animals were mated one male to one female for 17 days. The day that vaginal sperm or a copulation plug was observed was designated Day 0 of gestation. At Day 0 of gestation, the body weights ranged from 200 to 289 grams. Six groups were included in this study; for the purposes of this summary, only two groups (control, and BPA-DA-treated) will be discussed. Twenty-four animals per group were treated with vehicle (0.5% carboxymethyl cellulose) or BPA-DA (1000 mg/kg/day). Dose volume was 10.0 mL/kg. The dose was administered from gestation day (gd) 6 through 15, approximately the same time each day, and was based on the most recently recorded body weight. All of the animals were observed daily for mortality, moribundity and clinical signs. Body weights and food consumption were recorded on gd 0, 6, 8, 12, 16 and 20. On gd 20, the animals were sacrificed, examined for gross pathology of the external surface and viscera, and the uterus excised and weighed. The fetuses were taken by cesarean section and the following recorded for each litter: the number of corpora lutea per ovary; the number and placement of uterine implantation sites; live and dead fetuses; early and late resorptions; and any other abnormalities. Fetuses were removed from the placenta, individually identified, examined externally, and weighed.
Visceral Examination of Fetuses: Approximately one-third of the live fetuses were selected for visceral examination according to the method of Wilson.
Skeletal Examination of Fetuses: The remaining fetuses were eviscerated and placed in 95% ethyl alcohol. After fixation and dehydration, the skeletons were stained in a potassium hydroxide-alizarin red solution. The skull, vertebral column, rib cage, pectoral and pelvic girdles, long

bones and extremities of each skeleton were examined for degree of ossification, bone alignment, and possible anomalies.

Statistical Analyses: Mean maternal body weight changes, food consumption, percentage data (implantations, resorptions and males), and fetal viability were analyzed in the following order: Levene's test for homogeneity of variance; if the variances proved to be homogeneous, the data were analyzed by one-way classification analysis of variance (ANOVA); if the variance proved to be heterogeneous, a series of transformations was performed until homogeneity was achieved followed by ANOVA. If ANOVA was significant, the Dunnett's test was used to compare groups. Pregnancy rates, clinical observations and fetal skeletal observations were analyzed by Cochran-Armitage and Fisher-Irwin Exact Tests. Analysis of covariance (ANCOVA) was used to analyze mean fetal weights with the litter used as the experimental unit. Levene's test and ANOVA were evaluated at the 5% one-tailed probability level. Control vs. treatment group mean comparisons were evaluated at the 5% two-tailed probability level.

Range-finding study: A range-finding study was conducted to select the dose used in this study. Five pregnant Sprague-Dawley rats were dosed with BPA-DA at doses of 150, 400 or 1000 mg/kg/day from days 6 through 15 of gestation. A slight decrease in maternal body weight gain was observed at 1000 mg/kg/day. No fetal effects were observed at any dose. Based on this study, 1000 mg/kg/day was selected for the teratology study.

Results:

Mean body weight was significantly lower than the control group on gd 8, 12, 16 and 20 and mean weight gain was significantly lower than control for gd 6-16. Mean maternal food consumption was significantly lower than control for gd 6-8 and 8-12. No effects on gross pathology of the dams were observed.

The following tables summarize the fetal results:

Summary of Mean Ovarian, Uterine, and Litter Data

Parameter	Control (Vehicle)	BPA-DA (1000 mg/kg/day)
Number of dams	24	24
Number pregnant	23	24
Pregnancy rate (%)	96	100
Number dams surviving to gd 29 (survival rate)	24 (100%)	24 (100%)
Mean number of Corpora lutea	16.9	15.9
Implantations (% Efficiency)	14.8 (89)	14.3 (90)
Resorptions-total	0.9	0.4
Fetuses – live	14.0	13.8
– dead	0	0
Live fetuses		
Mean body weight (g) – males	3.6	3.5
– females	3.3	3.4
Mean uterine weights – gravid (g)	76.3	75.2

Summary of Mean Incidence of Abnormal Fetuses per Litter

Parameter	Control (Vehicle)	BPA-DA (1000 mg/kg/day)
External Variations		
Litter Incidence	23	24
# of litters examined	3	3
# of litters with anomalous fetuses	13	13
% of litters with anomalous fetuses		
Fetal Incidence	4	4
# of fetuses with variants	1.2	1.2
Incidence of variant (%)		
External Malformations		
Litter Incidence	23	24
# of litters examined	1	0
# of litters with anomalous fetuses	4.3	0
% of litters with anomalous fetuses		
Fetal Incidence	1	0
# of fetuses with variants	0.3	0
Incidence of variant (%)		

Summary of Mean Incidence of Abnormal Fetuses per Litter

Parameter	Control (Vehicle)	BPA-DA (1000 mg/kg/day)
Soft Tissue Variations		
Litter Incidence	23	24
# of litters examined	6	6
# of litters with anomalous fetuses	26	25
% of litters with anomalous fetuses		
Fetal Incidence	9	9
# of fetuses with variants	9.2	8.8
Incidence of variant (%)		
Soft Tissue Malformations		
Litter Incidence	23	24
# of litters examined	0	0
# of litters with anomalous fetuses	0	0
% of litters with anomalous fetuses		
Fetal Incidence	0	0
# of fetuses with variants	0	0
Incidence of variant (%)		
Skeletal Variations		
Litter Incidence	23	24
# of litters examined	23	23
# of litters with anomalous fetuses	100	96
% of litters with anomalous fetuses		
Fetal Incidence	120	120
# of fetuses with variants	54	52
Incidence of variant (%)		
Skeletal Malformations		
Litter Incidence	23	24
# of litters examined	1	0
# of litters with anomalous fetuses	4.3	0
% of litters with anomalous fetuses		
Fetal Incidence	1	0
# of fetuses with variants	0.4	0
Incidence of variant (%)		

Conclusion:

Mean body weight and weight gain were reduced for BPA-DA treated dams in this study. There were no differences from control in the BPA-DA dose groups for ovarian or uterine data. There were no treatment-related effects on any fetal parameters from BPA-DA treatment. Based on the results of this study, BPA-DA is not a developmental toxin.

- Reference: Morseth, S. L. (1987). Unpublished Report No. HLA 349-265 entitled “Rat Teratology Study with BPA-DA, BPA-BI and NMP”, dated March 5, 1987 for General Electric Company, Plastics Business Operations, Pittsfield, MA, USA; from Hazleton Laboratories America, Inc., Vienna, VA, USA; and
- Burdock, G.A. (1985). Unpublished Report No. 349-326 entitled “Pilot Rat Teratology Study: NMP, BPA-BI, BPA-DA, and a Positive Control”, dated August October 29, 1985 for General Electric Company, Pittsfield, MA, USA; from Hazleton Laboratories America, Inc., Vienna, VA, USA.
- Reliability: (Klimisch Code 1) Reliable without restrictions.