201-15483

Ciba Specialty Chemicals
Corporation

Richard Balcomb
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Ciba

July 27, 2004

Michael O. Leavitt
Administrator
US Environmental Protection Agency
HPV Challenge

Subject: Test Plan and Robust Summaries for IRGANOX 1330 / ETHANOX® 330
1,3,5-trimethyl-2, 4,6-tris (3,5-di-t-butyl-4-hydroxybenzyl) benzene
CAS No. 1709-70-2

Dear Mr. Leavitt:

Ciba Specialty Chemicals Corporation and Albemarle Corporation support EPA's High Production Volume (HPV) chemical challenge program and its effort to gather and publish basic hazard information on those chemicals manufactured at high volumes in the United States. Ciba Specialty Chemicals Corporation and Albemarle Corporation are co-sponsors for 1,3,5-trimethyl-2, 4,6-tris (3,5-di-t-butyl-4-hydroxybenzyl) benzene and we here submit for review and public comment our available data and test plan.

Sincerely yours,

Richard Balcomb

HPV Registration Number:

201-15483B

EPA High Production Volume Program

Test Plan for

IRGANOX 1330 / ETHANOX 330

1,3,5-trimethyl-2, 4,6-tris (3,5-di-t-butyl-4-hydroxybenzyl) benzene

CAS No. 1709-70-2

July 30, 2004

Submitted by:

Ciba Specialty Chemicals
540 White Plains Road
Tarrytown, New York, 10591
And
Albemarle Corporation
451 Florida Street
Balton Rouge, LA 70801

Executive Summary

A. Introduction

An important objective of EPA's High Production Volume (HPV) chemical challenge program is the gathering and public release of basic hazard information on chemicals imported or manufactured at high volumes in the United States. Ciba Specialty Chemicals and Albemarle Corporation have agreed to participate in this program and here submit for review and public comment the available data and test plan for Irganox 1330 / Ethanox 330.

B. General Substance Information

Chemical Name: 1,3,5-trimethyl-2, 4,6-tris (3,5-di-t-butyl-4-hydroxybenzyl) benzene

Appearance: White solid

Typical Commercial Purity: 98 – 100%

Chemical abstract Service Registry Number: CAS # 1709-70-2

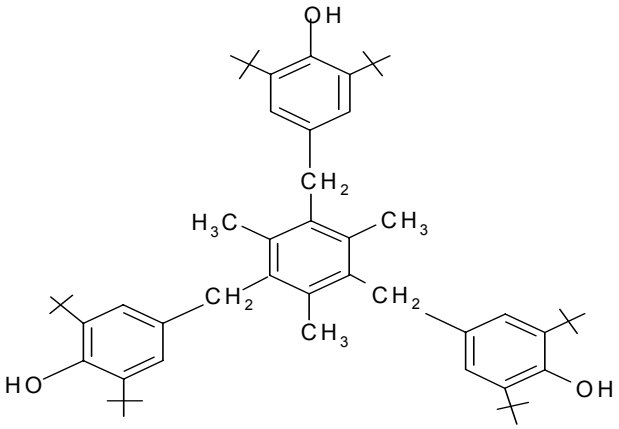
Trade Names: IRGANOX 1330 (Ciba)
ETHANOX 330 (Albemarle)

Other Synonyms / Trade Names: Ionox 330, Ethyl 330, Ethyl Antioxidant 330, AO 40

Chemical Formula: C54H78O3

Molecular weight: 775.22

Structure:



C. General Use Information

1,3,5-trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl)benzene, commercially known as Irganox 1330 and Ethanox 330, is a sterically hindered phenolic antioxidant that protects organic substrates against thermo-oxidative degradation.

Irganox 1330 / Ethanox 330 is used in polyolefins (e.g., polyethylene, polypropylene, polybutene) for the stabilization of pipes, molded articles, wires and cables, dielectric films and in other polymers such as engineering plastics like linear polyesters, polyamides, and styrene homo- and copolymers. It may also be used in PVC, polyurethanes, elastomers, adhesives, and other organic substrates.

This product has been regulated by the FDA for use in all polymers at a maximum level of 0.5% except for nylon resins where the maximum use level is 1%. The resultant polymers would conceivably contact all food types with no temperature restrictions.

Sales of Irganox 1330 / Ethanox 330 are to industrial users only. The polymer industry has a record of safe use of additives such as Irganox 1330 / Ethanox 330 and worker exposures are considered minimal. Industrial Hygiene programs and Responsible Care® practices are the norm throughout the industry and it is the experience of Ciba Specialty Chemicals and Albemarle Corporation that customers handle such products in a careful and conscientious manner. Material Safety Data Sheets (MSDS) are distributed that present detailed hazard data and provide directions for safe handling. After Irganox 1330 / Ethanox 330 is incorporated in the polymer matrix it is relatively immobile and release-exposure to humans or the environment is considered minimal.

Environmental Endpoints:

Ecotoxicology testing for Irganox 1330 / Ethanox 330 indicates the compound has low toxicity to fish, aquatic plants and aquatic invertebrates. Aquatic toxicity testing was conducted with water loadings well above the solubility limits of the compound; these test conditions provide a worst-case challenge to the test organisms and indicate that there is low concern for environmental effects. Furthermore, under environmental conditions the low solubility of the material (< 1mg/L) should preclude the occurrence of acutely toxic exposures. The compound has a calculated n-octanol-water coefficient (log Pow) of > 6. Based on this and its other physical-chemical properties, the substance in the environment is likely to bind to the soil and sediment where it is expected to be immobile and have limited bioavailability. The material is not readily biodegradable. Based on its present commercial use, environmental release and exposures are expected to be negligible.

Toxicology Endpoints:

Available mammalian acute toxicity data indicates the compound has very low toxicity by oral, dermal, or inhalation exposure. In a 28-day toxicity study in the rat, there are no adverse effects up to 1000 mg/kg bw per day. In subchronic 15-week toxicity studies with rats and dogs, there are no significant effects observed and a dietary NOEL greater than 5000 ppm was indicated. In 2-year carcinogenicity studies, neither tumors nor lesions were observed with dietary exposures ranging from 400 to 10,000 ppm. Repeat dose testing consistently shows that Irganox 1330 / Ethanox 330 does not have adverse effects on reproductive organs, even at relatively high exposure levels, and multi-generation reproduction testing did not demonstrate significant toxicity or teratogenic potential. Genetic toxicity testing has shown that the compound is not mutagenic. Chromosomal aberration testing is not available, however, multiple chronic studies, that are discussed in detail in the robust summary document, show that the compound does not induce tumors and is not carcinogenic. Therefore, there is no need to conduct a chromosome aberration test at this time. Furthermore, available genetic toxicity testing with other hindered phenols has indicated a low potential for mutagenic or claustogenic effects (see HPV Hindered Phenol submission, American Chemistry Council, 2003). All toxicological endpoints are fulfilled.

Conclusions

The available data are sufficient to meet the requirements of the HPV challenge program and no additional testing is proposed.

SUMMARY TABLE

CAS NO. 1709-70-2	DATE	RESULTS	FULFILLS REQUIREMENT
PHYSICAL/CHEMICAL ELEMENTS			
Melting Point	2003	240 - 245 ^{o}C	Yes
Boiling Point	2004	821.96 °C	Yes
Vapor Pressure	2004	3.14 x 10 $^{-22}$ mm Hg (estimated) 1.3 x 10^{-12} Pa (measured)	Yes
Partition Coefficient	2004 1988	log Kow > 17.17 (estimated) log Kow > 6.0 (measured)	Yes
Water Solubility	1992 2003	< 1 mg / liter (measured) 9.11 x 10^{-14} mg/ L (estimated)	Yes
ENVIRONMENTAL FATE AND PATHWAYS ELEMENTS			
Photodegradation	2004	For reaction with hydroxyl radical, predicted rate constant = 150.13 x 10^{-12} cm^3/molecule-sec. Predicted half-life = 0.855 hours	Yes
Stability in Water	2004	EPIWIN model could not evaluate this structure. Experimental determination is not practical due to low water solubility.	Waiver
Fugacity	2004	Predicted distribution using Level III fugacity model Air 0.0134 % Water 1.26 % Soil 32.8 % Sediment 66 %	Yes
Biodegradation	1988	Not biodegradable 10 mg/L: 6% in 28 days 20 mg/L: 16% in 28 days	Yes
ECOTOXICITY ELEMENTS			
Acute Toxicity to Fish	1988	Zebra Fish : LC_{50} (96 h) > 100 mg/L	Yes
Toxicity to Aquatic Plants	1988	EC_{50} (0-72 h) > 100 mg/L	Yes
Acute Toxicity to Aquatic Invertebrates	1988	EC_{50} (24 h) > 100 mg/L	Yes

SUMMARY TABLE (CONTINUED)

CAS No. 1709-70-2	DATE	RESULTS	FULFILLS REQUIREMENT
HEALTH ELEMENTS			
Acute Toxicity	1965	Rat: LD_{50} (Oral) > 5,000 mg/kg	Yes
	1992	Rabbit: LD_{50} (Dermal) > 2,000 mg/kg	
	1983	Rat: LD_{50} (Inhalation) > 1,000 mg/ m^3	
Genetic Toxicity • Gene Mutation	1984	Ames Test - Salmonella typhimurium: No increase in mutations with or without metabolic activation (at doses of 0, 0.05, 0.1, 0.2, 0.5, 1.0, and 2.0 mg/ plate)	Yes
• Chromosome Aberration		No testing available	Available chronic testing precludes the need for this study
Repeat Dose Toxicity • Subchronic Toxicity			
i) 15 Week oral toxicity study in rats	1966	NOEL > 5000 ppm	Yes
ii) 15 Week oral toxicity study in dogs	1966	NOEL >5000 ppm	
• Chronic Toxicity Carcinogenicity			
i) 2-Year Oral Toxicity Study in Rats and Mice	1969	NOEL = 5000 ppm No tumors or lesions were observed	Yes
ii) 2-Year Oral Toxicity Study in Dogs	1968	NOEL = 10,000 ppm No tumors or lesions were observed	
iii) 2-Year oral toxicity study in rats	1968	NOEL = 2000 ppm NOAEL = 10000 ppm No tumors or lesions were observed	
Reproductive and Developmental Toxicity	1970	No significant effects on reproduction or development in a three-generation study. NOEL = 5000 ppm	Yes

Robust Summaries for

IRGANOX 1330 / ETHANOX® 330

1,3,5-trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl)benzene

CAS No. 1709-70-2

Name and Address of Sponsoring Organizations:

Ciba Specialty Chemicals Corporation
540 White Plains Road
Tarrytown, New York 10591
Tel: (914) 785-2000
Fax: (914) 785-4147

Albemarle Corporation
451 Florida Street
Baton Rouge, LA 70801
Tel: (225) 388-7402
Fax: (225) 388-7848

HPV Registration Number:
Technical Contact Persons: Richard Balcomb and Shailaja Rao
Date: June 26, 2004

CONTENTS

HEALTH ELEMENTS

SUMMARY TABLE

CAS NO. 1709-70-2	DATE	RESULTS	FULFILLS REQUIREMENT
PHYSICAL/CHEMICAL ELEMENTS			
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Partition Coefficient	2004 1988	log Kow > 17.17 (estimated) log Kow > 6.0 (measured)	Yes
Water Solubility	1992 2003	< 1 mg / liter (measured) 9.11 x 10^{-14} mg/ L (estimated)	Yes
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Genetic Toxicity • Gene Mutation	1984	Ames Test - Salmonella typhimurium: No increase in mutations with or without metabolic activation (at doses of 0, 0.05, 0.1, 0.2, 0.5, 1.0, and 2.0 mg/ plate)	Yes
• Chromosome Aberration		No testing available	Available chronic testing precludes the need for this study
Repeat Dose Toxicity • Subchronic Toxicity			
i) 15 Week oral toxicity study in rats	1966	NOEL > 5000 ppm	Yes
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• Chronic Toxicity Carcinogenicity			
i) 2-Year Oral Toxicity Study in Rats and Mice	1969	NOEL = 5000 ppm No tumors, lesions were observed	Yes
ii) 2-Year Oral Toxicity Study in Dogs	1968	NOEL = 10,000 ppm No tumors, lesions were observed	
iii) 2-Year oral toxicity study in rats	1968	NOEL = 2000 ppm NOAEL = 10000 ppm	
Reproductive and Developmental Toxicity	1970	No significant effects on reproduction or development in a three-generation study. NOEL = 5000 ppm	Yes

General Substance Information

Chemical Name: 1,3,5-trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl)benzene

Appearance: White to slightly yellow solid

Typical Commercial Purity: >99%

Chemical Abstract Service Registry Number: 1709-70-2

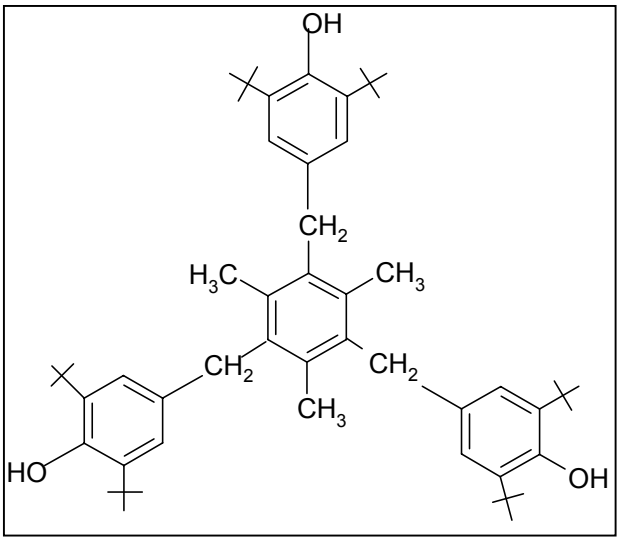
Trade Names: Irganox 1330 / Ethanox 330

Other Synonyms / Trade Names: Ionox 330, Ethyl 330, Ethyl Antioxidant 330, AO 40

Chemical Formula: $C_{54}H_{78}O_3$

Molecular weight: 775.22

Structure:



PHYSICAL/CHEMICAL ELEMENTS

1. MELTING POINT

Test substance:	1,3,5-trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl)benzene CAS No. 1709-70-2
Method:	Not reported[1]
GLP:	No
Year:	2003
Results:	240-245 °C
Remarks:	The melting point was reported in the MSDS (dated 12/16/2003) from Ciba Specialty Chemicals Corporation. The melting point was assigned a reliability code of 2g[2] (data from Handbook or collection of data).
References:	[1]Material Data Sheet from Ciba Specialty Chemicals, dated 12/16/2003. [2]Klimisch, H.J., Andreae, M and Tillman, U., A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

2. BOILING POINT

Test substance:	1,3,5-trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl)benzene CAS No. 1709-70-2
Method:	Since it is a solid, boiling point is estimated by the MPBPWIN Program (v. 1.40) using the adapted Stein and Brown Method).[1,2]
GLP:	No
Year:	2004
Results:	821.96 °C
Remarks:	In the absence of reliable experimental data, the boiling point was calculated using an accepted method and assigned a reliability code of 2f [3] (Accepted calculation method).
References:	[1]Syracuse Research Corporation, Syracuse, NY [2]Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998. [3]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

3. VAPOR PRESSURE

Test substance:	1,3,5-trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl)benzene CAS No. 1709-70-2
Method:	Estimated by the MPBPWIN Program (v. 1.40) using the modified Grain method.[1, 2]
GLP:	No
Year:	2004
Results:	3.14 x 10^{-22} mm Hg
Remarks:	A vapor pressure of 1.3 x 10^{-12} Pa was reported in the MSDS from Ciba Specialty Chemicals Corporation. Details of the testing for this determination are not available. In the absence of this information, the vapor pressure was calculated using an accepted method. The estimate was assigned a reliability code of 2f[3] (Accepted calculation method).
References:	[1]Syracuse Research Corporation, Syracuse, NY [2]Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998 [3]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

4. PARTITION COEFFICIENT

Test substance:	1,3,5-trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl)benzene CAS No. 1709-70-2
Method:	Experimental method[1] KOWWIN Program (v. 1.67)[2, 3]
GLP:	No
Year:	1988
Results:	Log Kow > 6.0 (experimental data) Log Kow > 17.17 (model data)
Remarks:	A partition coefficient of > 17.17 was also calculated by an accepted method (KOWWIN Program). Details of the testing for the experimental data are not available. In the absence of this information, the partition coefficient was calculated using an accepted method. The estimate was assigned a reliability code of 2f[3] (Accepted calculation method).
References:	[1]Partition Coefficient of TK 10455. Ciba-Geigy Ltd., Physical Chemistry Department, Basle, August 10, 1988. [2]Syracuse Research Corporation, Syracuse, NY [3]Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998. [4]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

5. WATER SOLUBILITY

Test substance:	1,3,5-trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl)benzene CAS No. 1709-70-2
Method:	The test conditions are adapted from the EEC directive 84/ 449 A.6. Test solution of 250 mg of Irganox 1330 in 500 ml Millipore water SQS HPLC quality was used. The saturation temperature was 30°C and the saturation time of 24 hours. The analytical method used was photometric determination at 285 nm.[1]
Temperature:	20 °C
Equilibrium Time:	24 hours
GLP:	No
Year:	1992
Results:	Solubility < 1 mg / liter
Remarks:	The water solubility was also calculated by an accepted method. The calculated value was 9.11×10^{-14} mg/L (WSKOW v1.41 [2, 3]) The estimate was assigned a reliability code of 2c[4] (Comparable to guideline study with acceptable restrictions).
References:	[1]Water Solubility of TK 10455. Ciba-Geigy, Analytical Department, Additives, Basel, Switzerland. Project Nr. 3982. August 17, 1992
	[2]Syracuse Research Corporation, Syracuse, NY
	[3]Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998.
	[4]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

ENVIRONMENTAL FATE AND PATHWAYS ELEMENTS

6. PHOTODEGRADATION

Test substance:	1,3,5-trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl)benzene CAS No. 1709-70-2
Method:	Estimated by the AOP program (v. 1.91). [1,2] This model estimates rate constants and half-lives of atmospheric reactions of organic compounds with hydroxyl radicals and ozone in the atmosphere.
GLP:	No
Year:	2004
Results:	For reaction with hydroxyl radicals, the predicted half-life of the chemical was rapid. Rate constant: 150.13 x 10^{-12} cm^3/molecule-sec Half-life: 0.855 h
Remarks:	In the absence of reliable experimental data, the photodegradation was calculated using an accepted method and assigned a reliability code of 2f [3] (Accepted calculation method).
References:	[1]Syracuse Research Corporation, Syracuse, NY [2]Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998 [3]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

7. STABILITY IN WATER / HYDROLYSIS

Test substance:	1,3,5-trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl)benzene CAS No. 1709-70-2
Method:	The HYDROWIN Program (v. 1.67) [1,2]
GLP:	No
Year:	2004
Results:	The HYDROWIN Program was unable to evaluate the fragments of this chemical structure. This material is of low water solubility.
Remarks:	The estimate was assigned a reliability code of 2f[3] (Accepted calculation method).
References:	[1]Syracuse Research Corporation, Syracuse, NY [2]Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998. [3]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

8. THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)

Test substance:	1,3,5-trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl)benzene CAS No. 1709-70-2
Method:	Estimated by EPIWIN Level III Fugacity Model.[1, 2]
Year:	2004
GLP:	No
Results:	Distribution using EQC Level III Fugacity Model

Air	0.0134 %
Water	1.26 %
Soil	32.8 %
Sediment	66 %

Persistence Time = 5.6×10^3 h

Remarks: In the absence of reliable experimental data, the fugacity was calculated using an accepted method and assigned a reliability code of 2f [3] (Accepted calculation method).

References:

[1]Syracuse Research Corporation, Syracuse, NY

[2]Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998.

[3]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

9. BIODEGRADATION

Test substance:	1,3,5-trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl)benzene CAS No. 1709-70-2
Method:	This study was conducted under OECD Guideline 301 B "Ready Biodegradability: Modified Sturm Test (CO_2 Evolution)," May 1981 and the EEC directive 79/831 Annex V Part C 5.2. Bacteria was collected from activated sludge of a sewage treatment plant. The preparation was carried out according to the guidelines, with the exception that the volume of test solution was reduced from 3 to 1.5 L. Two liter flasks equipped with gas inlet and magnetic stirrer were used. 1200 ml of the mineral solution with the inoculum were aerated for 24 hours in the test vessel. In 300 ml mineral solution 0.5 ml Nonylphenol 10E05P0 and 15 rsp. 30 mg of test substance were added and homogenized. This solution was given to the test vessel which was immediately connected to the CO2 traps. Due to the poor solubility of the test substance in water, an emulsifier was used to achieve a better distribution in the medium. The temperature was maintained at 22 ± 2° C, with air at approximately 25 ml/min free of carbon dioxide. [1]
Test Type:	Aerobic
Duration of the test:	28 days
Concentration of the chemical:	Test chemical: 10 mg/ L and 20 mg/ L. Reference chemical: aniline (Merck No.1261): 20 mg/ L
Blank:	Water as specified in the guideline
Blank + Vehicle:	Water as specified in the guideline containing 0.5 ml of the Nonylphenol solution
Inoculum:	Fresh sewage treatment plant sample (per guideline)
Medium:	Sewage sludge (per guideline)
GLP:	No
Year:	1988
Results:	Test chemical: 10 mg/L: 6% degradation in 28 days 20 mg/L: 16% degradation in 28 days Aniline Reference: 20 mg/L: 94.4 % in 28 days. Under the test conditions, no biodegradation was observed.
Conclusion:	Substance was not readily biodegradable according to OECD definition.
Remarks:	This study was assigned a reliability code of 1b [2] (comparable to a guideline study).
Reference:	[1]Report on the test for ready biodegradability of Irganox 1330 in

the modified Sturm test, Ciba-Geigy Ltd., Basle, Switzerland, November 18, 1988. Project 88 43 77.

[2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

ECOTOXICITY ELEMENTS

10. A. ACUTE TOXICITY TO FISH

Test substance:	1,3,5-trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl)benzene CAS No. 1709-70-2
Method:	This study was conducted under OECD Guideline No. 203 (Paris, 1984) static procedure. This study was performed as a limit test with a maximum loading of 100 mg/L (nominal). Glass aquarium (20 L) was filled with 15 litres of dechlorinated tap water (carbon filter). Hardness of water was 175 mg CaCO3/L. Temperature was maintained at 23 ± 1 ^{o}C. Test substance was distributed homogeneously into the water at calculated amounts. Small amounts of the test substance were observed at the surface of the test solutions at concentration of 100 mg/L (nominal) from the start of the test. Zebra fish were supplied by West Aquarium / D-3422 Lauterberg. 20 fish in test concentration, tested in 2 separate tanks and 10 fish per control were used. Fluorescent light was used for 16 hours daily. Oxygen, pH, temperature were measured daily. Values are measured at 0 and 96 hour exposure. Test was performed as limit test with concentration of 100 mg/L nominal. [1]
Type of test:	Static
Species:	Zebra fish (*Brachydanio rerio*)
No.of fishes:	20 fish in test concentration, tested in 2 separate tanks 10 fish in blank control 10 fish in vehicle control
Length:	36 mm (23 - 28 mm)
Weight:	0.15 g (0.12 - 0.19 g)
Loading:	0.1 g/L
Exposure period:	96 h
Feeding:	None
Adaptation:	24 hours/ no food 24 hours prior to exposure
Acclimatization:	33 days
Treatment:	None
Test Concentrations:	100 mg/ L

Controls:	Blank: water Vehicle: 90.2 mg THF; 0.8 mg alkylphenol-polyglykol-ether used for test concentration.
GLP:	No
Year:	1988
Results:	LC_0 (96 h) > 100 mg/L LC_{50} (96 h) > 100 mg/L LC_{100} (96 h) > 100 mg/L Mortalities in Blank : 0% Mortalities in Vehicle: 0% Mortalities in Treatment: 0% The symptoms and observations were similar between the test group and the control and vehicle group.
Remarks:	The study was conducted with maximum loading considerably above the water solubility of the test compound and represents a worst case exposure. No toxic effects were observed and no further testing is warranted. This study was assigned a reliability code of 1b [2] (comparable to a guideline study).
Reference:	[1]Report on the test for acute toxicity of TK 10455 to Zebra fish, Project No. 884378, Ciba-Geigy Ltd., Basel, Switzerland, November 29, 1988. [2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

11. TOXICITY TO AQUATIC PLANTS

Test substance:	1,3,5-trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl)benzene CAS No. 1709-70-2
Method:	This study was conducted under test guideline: 87/302/EEC page 89-94, Algal growth inhibition test. S*cenedesmus subspicatus* were the test organisms and the study was conducted in 100-mL Erlenmeyer flasks containing 50 mL of algae nutrient media or test solution. Calculated amounts of the stock solution were homogeneously distributed into the water to make the desired concentrations. The algae were then transferred into the flasks. The nominal test concentrations were at 1.23, 3.7, 11, 33 and 100 mg /L. Each test concentration was tested in 3 replicates and the blank control in 6. The water quality parameters like temperature and pH were measured in each test solution at test initiation. The temperature was continuosly measured and maintained at 23 ± 1^{o}C and with continuous illumination with cold white florescent light, pH was measured at 0 and 72 h exposure. Algal cell densities were measured at 24, 48, 72 hours on a TOA cell counter.[1]
Species:	Green Algae (*Scenedesmus subspicatus*)
Initial Cell Density:	7000 cells/ ml
Test Procedure:	Static
Age of Culture at Study Initiation:	3 days old
Test concentrations:	1.23, 3.7, 11, 33 and 100 mg/L (nominal)
Controls:	Blank: water Vehicle: 100 mg chloroform
Relicates:	Each test concentration –3 replicates Blank control – in 6
Exposure period:	72 h
Analytical monitoring:	No
GLP:	No
Year:	1992
Results:	EC_{50} (0-72 h) > 100 mg/L NOEC (0-72 h) = 100 mg/L Values are based on nominal concentrations.

Remarks:	The study was conducted above the water solubility of the test compound and represents a worst case exposure. No toxic effects were observed and no further testing is warranted. This study was assigned a reliability code of 1b [2] (comparable to a guideline study).
Reference:	[1]Report on the growth inhibition test of Irganox 1330 to green algae (Scenedesmus subspicatus); Project No. 928154, Ciba-Geigy, Ltd., Basel, Switzerland; December 17, 1992. [2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

12. ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Test substance:	1,3,5-trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl)benzene CAS No. 1709-70-2
Method:	This study was conducted under OECD Guideline No. 202 (Paris 1984). The study used 20 young daphnia (0-24 h old) per concentration and control (4 replicates of 5 daphnia each). Stock solution was prepared by mixing 4.0 g of test substance with 80 mg of alkylphenol-polyglycol-ethes and made up to 10 ml with THF. 24 hours before the start of the test, reproductive daphnia were separated from the young by sieving all individuals through a 800 um sieve. Young daphnia of 0-24 h age were retained for the test. Cultures of daphnia are maintained in glass vessels containing approximately 2.5 L of reconstituted water at 20 ±1^{o}C. Water was renewed partially 3 times weekly. At each renewal the daphnia were fed a suspension of green algae supplemented by a suspension of Tetramin extract in such quantities that the food was consumed after 24 h. Desired test concentrations were made from the stock solution by dissolving in water. The daphnia were then transferred into the beakers covered with watch glasses. Fluorescent lighting was for 16 hours daily and the temperature was maintained at 20 ±1^{o}C. Oxygen, pH, temperature were measured daily. Samples were analysed at 0 and 24 hour exposure. Small parts of the test substance were observed at the surface of the test solutions at concentrations of 10-100 mg/L (nominal) from the start of the test.[1]
Species:	*Daphnia magna Straus 1820*
No. of daphnia:	20 daphnia per concentration and control 4 replicates of 5 daphnia each
Feeding:	None during the test
Type of test:	Static
Test concentration:	10, 18, 32, 58, 100 mg/L
Controls:	Blank: Water Vehicle: 133 mg THF and 0.2 mg alkylphenol polyglycol ether per litre water (the concentration used for the highest test concentration)

Water:	Reconstituted water produced by dissolving: 65 mg NaHCO3 294 mg CaCl2; 2 H2O 123 mg MgSO4; 7 H2O 6 mg KCl in 100 ml bidistilled water. Total hardness : 240 mg CaCO3/L The water was aerated with clean air for at least 24 h before use.
Exposure period:	24 hours
Temperature:	20 ±1°C
Analytical monitoring:	Yes
GLP:	No
Year:	1988
Results:	EC_{50} (24 h): > 100 mg/L EC_0 (24 h): > 100 mg/L EC_{100} (24 h): > 100 mg/L Values are based on nominal concentrations. Immobilization in blank and vehicle = 0%
Remarks:	The exposure concentrations exceeded the water solubility of the test substance and the concentrations were maximized by the use of solvents. These results represent a worst case exposure. This study was assigned a reliability code of 1b [2] (comparable to a guideline study).
Reference:	[1]Test for acute toxicity of TK 10455 to Daphnia magna, Project No.: 884379, Ciba-Geigy Ltd., Basle, Switzerland, November 17, 1988. [2] Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

HEALTH ELEMENTS

13. ACUTE TOXICITY

A. Oral

Test substance:	1,3,5-trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl)benzene CAS No. 1709-70-2
Method:	Rats bred within this laboratory from a strain originally derived by the hybridisation of the Carworth "E" and "N" strains were used. All animals were maintained at 23 ± 2 ^{o} C and a relative humidity of 45-50%. For acute oral dosing experiments, 2 male and 2 female rats were caged together for each dose level employed. The animals were fasted overnight prior to weighing and dosing. All the animals were observed frequently during the immediate post dosing period and for a further 10 days, after which time they were killed and autopsied. Groups of 2 male and 2 female rats were given single doses of 3.2, 4.0, or 5.0 g/kg of test substance as a 20% (w/v) solution in acetone-dimethylsulfoxide.[1]
Species/strain:	Rat, Carworth E and N strains
No. Animals/Group:	2 males and 2 females / dose level
Dose:	3.2, 4.0 or 5.0 g as a 20% (w/v) solution
Vehicle:	acetone-dimethylsulfoxide
Administration:	oral
GLP:	No
Year:	1965
Results:	LD_{50} (rats) > 5000 mg /kg bw One rat died at the highest dose level. This sole death occurred 2 days after dosing and no specific cause of death was determined on autopsy. Higher doses were not employed since the deaths occurring when volumes greater than 20 mg/kg solution are administered are often related to gastric distension. It is probable that the death which did occur was at least partly due to the large volume administered. It was therefore concluded that the acute oral LD50 of Ionox 330 to rats was > 5g /kg.

Remarks:	The study was assigned a reliability code of 2e[2] (meets generally accepted scientific standards, well documented and acceptable for assessment).
Reference:	[1]Toxicological studies with 2,4,6-Tri(3',5'-di-tert-butyl-4'-hydroxybenzyl)mesitylene in the Rat; D.E. Stevenson, P.L. Chambers, and C.G. Hunter; Tunstall laboratory, Shell Reasearch Ltd.; Food and Cosmetics Toxicology Vol. 3, pp. 281-288, 1965. [2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

B. Dermal

Test substance:	1,3,5-trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl)benzene CAS No. 1709-70-2
Method:	This study was conducted under OECD Guideline No. 402, adopted February 24, 1987. Young adult albino rats of both sexes were housed individually in Macrolon cages type 3, with standardized soft wood bedding. The animal room was air conditioned and the temperature is maintained at 22 ± 3 ^{o} C, relative humidity at 55 ± 15%, with 12 hours of lighting/day and approximately 15 air changes / hour. Rat food, NAFAG No. 890, NAFAG AG, Gossau, SG (Switzerland), and water were provided ad libitum. They were acclimatized for at least 5 days before exposure. The dose group consisted of 10 rats (5 males and 5 females). 24 hours prior to the dermal application, skin on the backs of the rabbits was shaved. The test article was evenly dispersed on the skin. It was covered with a gauze-lined semiocclusive dressing fastened around the trunk with an adhesive elastic bandage. After 24 hours the dressing was removed and the skin reaction was appraised repeatedly. Physical condition and rate of deaths were monitored throughout the observation period of 14 days.[1]
Species/strain:	Young adult albino rats
Total number of animals:	10
Frequency of application:	One dermal application
Exposure period:	24 hours
Post exposure observation period:	14 days
Dose levels:	2000 mg/kg body weight
Vehicle:	0.5% carboxymethylcellulose and 0.1% aqueous polysorbate 80.
Volume Applied:	4 g (corresponding ~ to 4 ml)
Year:	1992
Results:	LD50 > 2000 mg/kg body weight.. There were no mortalities observed. Piloerection and hunched posture were seen, being common symptoms in acute dermal tests. The animals recovered within 2 days. At necropsy, no deviation from normal morphology were found.
Remarks:	This study was assigned a reliability code of 1a[2] (guideline study).

Reference:

[1]Acute Dermal Toxicity Study in the Rat, June 22, 1992; Test No. 924061, Ciba-Geigy Ltd., Basel, Switzerland,

[2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

C. Intraperitoneal

Test substance:	1,3,5-trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl)benzene cinnamate) CAS No. 1709-70-2
Method:	The rats were caged in groups of 5 in Macrolon cages (type 3) with standardized soft wood bedding. The animals were allocated to the different dose groups by random selection. Prior to dosing, the animals were fasted overnight. The test substance, one single dose was injected into the peritoneal cavity. The animal room was air conditioned and the temperature is maintained at 22 ± 3 ^{o} C, relative humidity at 55 ± 15%, with 12 hours of lighting/day and approximately 15 air changes / hour. Rat food, NAFAG No. 890, NAFAG AG, Gossau, SG (Switzerland), and water were provided ad libitum. Physical condition and rate of deaths were monitored throughout the observation period of 14 days.[1]
Species/strain:	Rat, Tif:RAIf (SPF), F3-crosses of RII 1/Tif x RII 2/Tif
No. Animals/Group:	5 males and 5 females / dose level
Initial Body weight range:	193 – 235 g
Initial Age:	7- 8 weeks
Dose:	1000 mg / kg
Vehicle:	Distilled water containing 0.5% carboxymethylcellulose and 0.1% polysorbate 80.
Administration:	By intraperitoneal injection
Observation Period:	14 days or until all symptoms have disappeared
GLP:	No
Year:	1983
Results:	LD_{50} (rats) > 1000 mg /kg bw There were no mortalities observed. Dyspnoea, exophthalmus, ruffled fur and curved body position were sees, being common symptoms in acute tests. No specific symptoms were seen. There was practically no acute toxicity when administered intraperitoneally to the albino rats.

Remarks:	The study was assigned a reliability code of 2e[2] (meets generally accepted scientific standards, well documented and acceptable for assessment).
Reference:	[1]Acute Intraperitoneal LD_{50} in the Rat, Ciba-Geigy Ltd., October 25, 1983. GU Project No. 831157. [2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

14. GENETIC TOXICITY (Gene Mutation)

i) Bacterial Mutagenicity Test

Test substance:	1,3,5-trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl)benzene CAS No. 1709-70-2 Batch: EN 136168.04
Method:	A toxicity test was carried out with strains S.typhimurium TA 100 and E.coli WP2 uvrA without and with metabolic activation at six concentrations of the test substance (20.6 – 5000 ug/plate) and a negative control. The mutagenicity test was performed with S.typhimurium strains TA 98, TA 100, TA 1535 and TA 1537 and E.coli strain WP2 uvrA with and without metabolic activation. The plates were inverted and incubated for about 48 hours at 37 ± 1.5 ^{o}C in darkness. Thereafter, they were evaluated by counting the number of colonies and determining the background lawn.[1]
Type:	Bacterial mutagenicity screening test
System of testing:	Salmonella typhimurium TA 98, TA 100, TA 1535, and TA 1537, and E.coli WP2 uvrA
Concentrations:	Range in the cytotoxicity test 20.6 – 5000 ug/plate Range in the mutagenicity test 625 – 5000 ug/plate (with and without metabolic activation)
Vehicle:	Acetone
GLP:	No
Year:	1992
Results:	From the results of toxicity test, the highest concentration suitable for the mutagenicity test was selected to be 5000 ug/plate with and without metabolic activation. In the mutagenicity test normal background growth was observed with all strains at all concentrations. The numbers of revertant colonies were not reduced. The test substance exerted no toxic effect on the growth of the bacteria.
Remarks:	This study was assigned reliability code of 2e[2] (Meets generally accepted scientific standards, well documented and acceptable for assessment).
References:	[1]Bacterial Mutagenicity Screening Test. Genetic Toxicology, Ciba-Geigy Ltd., Basle, Switzerland; July 21, 1992. [2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

ii) Bacterial Mutagenicity Test

Test substance:	1,3,5-trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl)benzene CAS No. 1709-70-2
Method:	Test material dissolved in DMSO and emulsified with acetone containing 10% Tween. The material was tested for mutagenic effects on histidine-auxotrophic mutants of Salmonella typhimurium (TA 97, TA 98, and TA 100,) and Escherichia coli (WP2/pKM101). The investigations were performed with and without microsomal activation. The test was conducted at concentrations of 0, 0.05, 0.1, 0.2, 0.5, 1.0, and 2.0 mg/ plate.[1]
Type:	Bacterial mutagenicity
System of testing:	Salmonella typhimurium TA 98, TA 100, and E.coli WP2/pKM101
Concentrations:	0, 0.05, 0.1, 0.2, 0.5, 1.0, and 2.0 mg/ plate
GLP:	No
Year:	1984
Results:	In the range of testing concentration, cell toxicity was only observed with WP2/pKM 101. No increase of the frequency of mutant colonies was detected. No mutagenic potential could be attributed to the substance.
Conclusion:	The substance is not mutagenic.
Remarks:	This study was assigned reliability code of 2e[2] (Meets generally accepted scientific standards, well documented and acceptable for assessment).
References:	[1]Test for Mutagenic Properties in Bacteria with Plastic Additive Irganox 1330. Akita University, Dept. Medicine, Japan. 1984.
	[2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

15. GENETIC TOXICITY (chromosomal aberration)

Test substance:	1,3,5-trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl)benzene CAS No. 1709-70-2
Comment:	No study is available. A chromosomal aberration test has not been conducted. Availability of multiple chronic studies which are discussed in detail in this document (see section 17) shows that the compound does not induce tumors and is not carcinogenic.

16. REPEATED DOSE TOXICITY

A. Subchronic Toxicity:

i) 15 Week Oral Toxicity study in Rats:

Test substance:	1,3,5-trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl)benzene CAS No. 1709-70-2
Method:	This study was carried out over a 15-week period. Forty four, weaning albino rats were culled from a larger population after examination of each animal for general health and adequacy for testing purposes. The animals were equally divided as to sex and their body weights. All animals were housed individually in wire-bottomed cages. Animals in the experimental group were fed a daily diet containing 5000 ppm of test substance. Control group were given a diet containing 12% lard but without added test material. Diets were prepared from pulverized Rockland rat diet and distributed on a weekly basis. Both food and water were offered ad libitum. Behavioral patterns, body weight gains, survival, food consumption and clinical chemistry were evaluated. An histopathological examination was carried out on the following organs: spleen, mesenteric lymph node, cervical lymph node, sternum, skeletal muscle, trachea, lung, heart, submaxillary salivary gland, liver, pancreas, oesophagus, stomach (cardia, fundus and pylorus), small intestine (duodenum, jejunum, and ileum), caecum, colon, kidney, urinary bladder, prostate, seminal vesicle, testis, uterus, ovary, pituitary gland, adrenal gland, thyroid with parathyroid gland, thymus, peripheral nerve, brain, sternum, skeletal muscle. Organ weights of liver, kidneys, spleen, gonads, heart, and brain were taken. Microscopic pathologic studies were conducted on tissues and organs taken.[1]
Species/strain:	Albino rats
Initial Body weight:	60 – 75 g
No. of animals:	44 rats in total 10 males and 10 females/ control group 12 males and 12 females/ test group
Route of administration:	Oral in the diet
Exposure period:	90 days
Dose:	0 and 5000 ppm in food
GLP:	No

Year: 1984

Results: Body weight gains, food consumption, specific food consumption in relation to body weight, water consumption were similar to that of the control group. No mortalities occurred. No clinical symptoms and no signs of local and / or systemic toxicity were observed.

Hematologic and clinical chemistry studies, urine studies showed no significant abnormalities.

Organ Weights and Ratios: Mean organ weight data with statistics of standard deviation and range for each organ are presented in the following summary table. Organ weight data derived from the experimental animals were considered to be comparable to those obtained from control animals.

Table 1

Organ Weights Data for Male Rats (in grams)

Organs	Group	Mean	Standard Deviation	Range
Liver	Control	20.9	1.3	18.6-22.7
	Test group	19.4	2.5	16.3-23.0
Kidney	Control	3.53	0.26	3.09-3.92
	Test group	3.42	0.53	2.44-3.88
Spleen	Control	0.69	0.06	0.60-0.79
	Test group	0.76	0.16	0.54-1.10
Testis	Control	3.72	0.38	3.25-4.31
	Test group	3.72	0.25	3.36-4.28
Heart	Control	1.44	0.12	1.29-1.59
	Test group	1.39	0.13	1.20-1.55
Brain	Control	2.19	0.09	2.01-2.31
	Test group	2.11	0.09	1.99-2.26

Table 2

Organ Weight Data for Female Rats (in grams)

Organs	Group	Mean	Standard Deviation	Range
Liver	Control	11.3	0.9	10.3-12.7
	Test group	11.0	1.5	8.9-13.9
Kidney	Control	2.13	0.15	1.87-2.38
	Test group	2.08	0.24	1.77-2.48
Spleen	Control	0.52	0.10	0.37-0.73
	Test group	0.51	0.09	0.37-0.67
Heart	Control	0.92	0.06	0.80-1.01
	Test group	0.94	0.10	0.80-1.12
Brain	Control	1.99	0.07	1.86-2.10
	Test group	1.98	0.14	1.72-2.19

Microscopic changes seen in the tissues and organs obtained from the control animals and the experimental animals are shown in the following table. None of the changes noted were considered to be related to the ingestion of the test material.

Table

Histopathological Changes

Organs	Group	Number of Animals Examined	Findings	Incidence	Average Grade
Lung	Control	5 Males 5 Females	Pneumonitis	5 4	+ +
	Test group	5 Males 5 Females	congestion	1 0	++ -
	Test group	5 Males 5 Females	Pneumonitis	5 4	+ +
Liver	Control	5 Males 5 Females	Pericholangitis	2 2	+ +
	Test group	5 Males 5 Females	Pericholangitis	0 1	- +
Kidney	Control	5 Males 5 Females	Nephritis	1 0	+ -
	Test group	5 Males 5 Females	Nephritis	2 0	+ -
Trachea	Control	5 Males 5 Females	Inflammation	1 1	+++ ++
	Test group	5 Males 5 Females	-	-	
Prostate	Control	5 Males			
	Test group	5 Males	Inflammation	1	+

Grading System:
- = negative
± = minimal
+ = slight
++ = mild
+++ = moderate
++++ = severe
+++++ = extreme

Conclusions: This study did not show treatment related effects at a dietary exposure of 5000 ppm.

Remarks: This study was assigned a reliability code of $2e^2$ (Meets generally accepted scientific standards, well documented and acceptable for assessment).

Reference: [1]Fifteen Week Subacute Oral Toxicity Study in Rats, Final Report, September 01, 1966. Lifestream Laboratories project no. 15.

[2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

ii) 15-Week Oral Toxicity study in Dogs:

Test substance:	1,3,5-trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl)benzene CAS No. 1709-70-2
Method:	In this study, 14 pure-bred beagle hounds of approximately 6 months old were kept in kennels equipped with outside runs. Dogs of the same sex and group were housed in a single kennel. Experimental group had four males and four females. Where as control group consists of three dogs per sex. The dietary levels of the test substance was 5000 ppm. One male and one female dog were separated from the original group of eight experimental animals after they had been on test for 90 days. These dogs were removed in preparation for a special radiotracer experiment. All dogs were under daily observation of a veterinarian through out the entire testing period to ascertain general health of the animals and to detect any signs or symptoms of toxicity. The daily ration for each dog consisted of 440 g of Wayne dog food "krums" and 60 g of pure lard. In addition each test animal received 5000 ppm of test material mixed into the daily diet. Food was made available to the dogs daily for two hours after which time the unconsumed food was collected and food consumption calculated. Water was available to all dogs at all times during the study. Each animal was weighed on the first day of the test and weekly once. Blood and urine samples were analyzed at 10, 35, and 88 days of the 15-week feeding study. Pathological studies were conducted. In addition, the weights of the brain, gonads, heart, kidneys, liver, and spleen of each dog were recorded. Microscopic examination was conducted on tissues taken from dogs of each sex from the control group and the exposure group. The following tissues and organs were processed, stained and examined: heart, aorta, trachea, lungs, liver, gall bladder, pancreas, esophagus, stomach, small intestine, caecum, colon, spleen, lymph node, kidney, urinary bladder, testis, ovary, prostate, uterus, salivary glands, adrenal glands, thyroid gland, parathyroid glands, pituitary gland, brain, spinal cord, peripheral nerves, bone marrow, and skeletal muscle. [1]
Species/strain:	Pure-bred Beagle dogs
No. of animals per group:	Test group: 4 males and 4 females; Control: 3 males and 3 females Total: 14 dogs
Route of administration:	Dietary
Exposure period:	90 days
Dose:	0 and 5000 ppm in food
GLP:	No

Year: 1966

Results: The general behavior of the animals in test group was comparable to the control group. Body weight gains and health remained normal in control and test group animals.

No changes were attributed to the test material in any of the following parameters: body weight (growth), food consumption, food utilisation, mortality, behavioral reactions, hematologic studies, clinical blood chemistry studies, and urine analysis.

No outstanding differences were noted between test and control dogs upon gross pathological examination. Focal pneumonitis was seen in most experimental and control dogs and subcapsular granuloma was noted in the kidney and the liver of a few animals from each group. No other noteworthy observations were recorded during the gross pathologic examinations.

Organ weight and Organ to Body weight and Organ to Brain weight ratio data did not reveal any significant abnormalities.

Microscopic evaluation showed changes in the tissues and organs which are present in most instances among both the control and test animals. No significant alterations were noted. Histopathological changes are shown in the table below.

Table

Histopathological Changes

Organs	Group	Number of Animals Examined	Findings	Incidence	Average Grade
Lung	Control	3 Males 3 Females	Pneumonitis	1 2	+ +
	Test group	3 Males 3 Females	Pneumonitis	1 0	+ -
Liver	Control	3 Males 3 Females	Pericholangitis	0 1	- +
	Test group	3 Males 3 Females	Pericholangitis	0 1	- +
Lymph Node	Test group	3 Males 3 Females	Hyperplasia	1 0	++ -
Prostate	Test group	3 Males	Inflammation	1	+
Kidney	Control	3 Males 3 Females	Parasitic Granuloma	0 1	- no grade
Colon	Control	3 Males 3 Females	Inflammation	0 1	- +
Gall Bladder	Control	3 Males 3 Females	Inflammation	0 1	- +

Conclusions:	This study did not show treatment related effects at a dietary exposure of 5000 ppm.
Remarks:	This study was assigned a reliability code of $2e^2$ (Meets generally accepted scientific standards, well documented and acceptable for assessment)
Reference:	[1]Fifteen-week sub-acute oral toxicity study of Ionox 330 in beagle dogs, Final Report, August 08, 1966. Project No.14, Lifestream laboratories, Inc., Illinois. [2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

17. CHRONIC TOXICITY/ CARCINOGENICITY

i) 2-Year Oral Toxicity Study in Rats and Mice:

Test substance:	1,3,5-trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl)benzene CAS No. 1709-70-2 Batch 89/5/6232, purity 98%
Method:	In this study, 240 rats and 192 mice were used. The animals were bred and maintained under specific-pathogen-free conditions. Groups of rats and mice, 30 males and 30 females, aged five weeks were individually caged. Diets containing 1000, and 5000 ppm Ionox 330 were provided. Control groups received untreated powder diet, Diet 86 powder. The animals were allocated at random to the treatment groups. Daily observations were made on the general health and behavior of all animals. Body weight and food intake were recorded weekly for the first 13 weeks of the experiment and thereafter at 4 weeks intervals. At autopsy, performed on all animals dying or killed during the experiment, a gross pathological examination was made. Histopathological examination was made on the following organs: liver, thyroid, parathyroid, heart, lungs, spleen, kidneys, adrenals, small and large intestine, stomach, pancreas, skin, skeletal muscle, salivary glands, lymph nodes, bladder, testes, prostate, uterus, ovaries, fallopian tubes, eye, and brain. Statistical analyses of body and organ weights were made using the initial body weight as a covariate in covariance analysis.[1]
Species/strain:	Rats: Carworth Farm E strain Mice: Carworth Farm No. 1 strain
Age:	5 weeks old
No. of animals:	240 rats in total 30 males and 30 females / test group 40 males and 40 females/ control group 192 mice in total 30 males and 30 females / test group 36 males and 36 females/ control group
Route of administration:	Oral
Exposure period:	2 years

Dose: 0, 1000, 5000 ppm

GLP: No

Year: 1969

Result: The general health and behavior of the treated and control groups of both rats and mice were similar. The morbidity of the rats and mice remained unaffected by the feeding of Ionox 330.

In rats, the only effect on body weight was in the 5000 ppm males during week 78 when a reduction occurred. In mice, no body weight changes were noted throughout the study. During the final six months of the experiment a loss of body weight in all the rats and mice occurred, but this loss appeared to be an effect of age rather than of treatment. Table 1 and 2 shows the details of body weights in rats and mice. In the rats, no changes in the food intake were noted. In mice, the increased food intake during week 26 was not considered related to the feeding of Ionox 330. No other effects occurred.

Table 1

Body Weights of Rats receiving Ionox 330 for two years

	Dietary Conc (ppm)	Body Weight (g) at Week								
Males		0	13	26	39	52	64	78	92	104
	0	146	407	457	476	479	485	481	452	420
	1000	142	404	448	473	471	484	480	452	420
	5000	142	407	450	466	470	474	463	444	397
	Standard error of treatment Mean	± 2.2 ± 2.5	± 3.8 ± 4.5 ± 4.4	± 4.1 ± 4.9 ± 4.8	± 4.6 ± 5.5 ± 5.2	± 3.7 ± 4.5 ± 4.3	± 4.1 ± 4.9 ± 5.1	± 4.8 ± 5.5 ± 5.6	± 5.5 ± 5.6 ± 5.9	± 8.4 ± 7.5 ± 9.3
Females										
	0	121	273	313	335	343	354	360	357	326
	1000	123	269	307	328	333	345	348	350	317
	5000	126	275	311	331	331	346	350	353	314
	Standard error of treatment Mean	± 1.5 ± 1.8	± 2.7 ± 3.1	±3.1 ± 3.7 ±3.6	±3.5 ±4.1 ±4.0	±3.5 ±4.0 ±3.9	± 5.4 ± 6.2 ± 6.0	± 5.1 ± 5.9 ± 5.7	± 5.6 ± 6.5 ± 6.4	± 6.0 ± 6.3 ± 6.5

Treatment means adjusted for differences in the initial body weight.
≤ 0.05 Significance of the difference between treatment and control mean

Table 2

Body Weights of Mice receiving Ionox 330 for two years

	Dietary Conc (ppm)	Body Weight (g) at Week								
Males		0	13	26	39	52	64	78	92	104
	0	23.8	31.7	34.9	39.3	39.4	39.9	40.8	42.0	36.1
	1000	24.5	31.9	35.3	39.9	39.8	41.4	42.3	43.7	35.8
	5000	24.0	32.0	36.3	39.6	39.8	40.8	41.8	43.5	
	Standard error of treatment Mean	± 0.52 ± 0.57	± 0.45 ± 0.49	±0.47 ± 0.51	± 0.51 ± 0.55	± 0.51 ± 0.55	± 0.61 ± 0.67 ± 0.68	± 0.77 ± 0.83 ± 0.88	± 0.68 ± 0.80 ± 0.81	± 1.13 ± 1.35 ± 1.32
Females										
	0	20.2	28.6	32.3	33.6	33.9	35.0	34.6	35.5	32.9
	1000	19.7	27.6	31.8	33.7	34.5	34.8	35.7	35.9	33.7
	5000	19.6	27.8	30.8	32.7	33.2	33.3	33.2	35.4	30.3
	Standard error of treatment Mean	± 0.29 ± 0.31	± 0.36 ±0.40	± 0.45 ±0.51 ± 0.50	± 0.54 ± 0.63 ±0.60	± 0.52 ± 0.59 ± 0.57	± 0.73 ± 0.83 ± 0.80	± 0.78 ± 0.90 ± 0.84	± 0.93 ± 1.02	± 1.03 ± 1.17 ± 1.12

Treatment means adjusted for differences in the initial body weight.
≤ 0.05 Significance of the difference between treatment and control mean

Pathological examination of the tissues did not reveal any lesions attributed to the test substance. No evidence of any carcinogenic effect was obtained and no differences in the tumor incidence in treated and control animals.

The most common lesion in the rats was nephrosis. Renal lesions were found in virtually all the rats killed at the end of the trial. In the animals killed at the end of the exposure period the most common tumor present in rats was a thyroid adenoma and the next most common a basophil adenoma of the anterior pituitary. Table 3 shows the distribution of lesions and tumors in rats.

Table 3

Distribution of Lesions and Tumors in Rats

Dietary Conc. (ppm) ConcentraCo	No. of Rats	Lesions						Tumors							No. of Rats with Tumors	% tumor incidence
		Kidney	Liver	Heart	Alim. Tract	Lung	Adrenal	Thyroid	Mammary	Ant. Pit.	Adrenal	Skin	Lymph Node	Others		
a) Dying or Killed during experiment																
Males:																
0	24	12	1	5	2	0	0	3	0	1	2	1	1	1	8	33
1000	10	5	0	0	0	1	0	1	0	0	0	1	0	0	2	20
5000	16	6	1	4	0	1	1	1	0	1	0	1	0	1	3	19
Females:																
0	18	10	1	2	1	0	1	5	7	0	2	0	0	1	11	61
1000	10	3	0	0	0	2	0	1	2	1	2	0	1	0	4	40
5000	11	4	2	1	1	0	1	1	4	2	0	0	1	0	4	36
a) Killed after 2 years																
Males:																
0	16	16	8	1	4	0	7	6	0	3	2	1	1	2	7	44
1000	20	18	7	6	0	3	4	6	0	2	1	0	0	2	9	45
5000	14	14	4	5	1	0	3	6	0	2	1	0	0	0	7	50
Females:																
0	22	22	9	3	4	1	7	8	4	3	0	0	0	4	10	45
1000	20	19	9	5	1	4	9	6	3	5	0	0	0	2	15	75
5000	19	19	8	4	3	2	10	8	2	3	0	0	0	3	12	63

In conclusion, Ionox 330 at a dietary concentrations of 1000 and 5000 ppm did not produce any treatment-related toxicological effects or changes of a carcinogenic nature. The NOEL was considered to be 5000 ppm.

Remarks:	This study was assigned a reliability code of 2e[2] (comparable to guideline study with acceptable restrictions).
Reference:	[1]Studies on the oral toxicity of Ionox 330, Carcinogenesis study with rats and mice. Shell Research Ltd. London, Tunstall Laboratories, Sittingbourne, UK. Project No. T507190/1. March 1969 [2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

ii) 2-Year Oral Toxicity Study in Dogs:

Test substance:	1,3,5-trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl)benzene CAS No. 1709-70-2 Batch 891516232, purity 98%
Method:	In this study, 26 beagle dogs were used. The animals were bred at the laboratory where the experiment was conducted. The dogs were inoculated at three months. Groups of four males and four females, aged five months received diet containing 2000, and 10000 ppm Ionox 330. Control groups received untreated powder diet. The animals were allocated at random to the treatment groups. Daily observations were made on the general health and behavior of all animals. Body weight and food intake were recorded weekly. After six and 12 weeks exposure and there after at three monthly intervals. Haematological examination carried out on all blood samples comprised haemoglobin and packed cell volume determinations and erythrocyte, leucocyte and differential leucocyte counts. Clinical chemical determinations were made of serum urea and total protein. Urine examination and bromsulphthalein (BSP) liver function tests were carried out on the control and top dose groups every four months. At autopsy, performed on all animals dying or killed during the experiment, a gross pathological examination was made. Histopathological examination was made on the following organs: liver, thyroid, parathyroid, heart, lungs, spleen, kidneys, adrenals, small and large intestine, stomach, pancreas, skin, skeletal muscle, salivary glands, lymph nodes, bladder, testes, prostate, uterus, ovaries, fallopian tubes, eye, and brain.[1]
Species/strain:	Beagle dogs bred at that laboratory
Age:	5 months old
No. of animals:	26 Beagle dogs in total 4 males and 4 females / test group 5 males and 5 females/ control group
Route of administration:	Oral
Exposure period:	2 years
Dose:	0, 2000, 10000 ppm
GLP:	No
Year:	1968

Result: The general health and behavior of the treated and control groups were similar. The body weights of the dogs remained unaffected by the feeding of Ionox 330 (Table 1)

Table 1 - Body weights of dogs fed Ionox 330 for two years

Dietary Concn (ppm)	Number of Animals	Body weight (kg) at week								
		0	13	24	38	52	68	80	92	104
Males			+	+	+	+				
0	5	7.7	11.3	12.2	12.5	12.8	13.6	14.0	14.3	14.6
2000	4	8.0	11.3	12.5	12.7	13.2	14.3	14.8	14.8	15.0
10000	4	7.3	11.6	12.9	13.0	13.2	13.5	14.0	14.2	14.5
S.E. of a treatment mean	5	± 0.22	± 0.17	± 0.28	± 0.37	± 0.38	± 0.45	± 0.57	± 0.56	± 0.58
	4	± 0.24	± 0.19	± 0.32	± 0.41	± 0.42	± 0.51	± 0.63	± 0.63	± 0.65
Females			+							
0	5	6.7	10.2	10.6	11.0	11.4	11.9	12.2	12.2	12.3
2000	3	8.3	10.0	10.6	11.1	11.6	12.1	12.4	12.7	12.8
10000	3	7.7	10.3	11.3	11.7	11.9	12.4	12.6	12.6	13.1
S.E. of a treatment mean	5	± 0.71	± 0.19	± 0.28	± 0.37	± 0.35	± 0.38	± 0.42	± 0.41	± 0.42
	3	± 0.92	± 0.25	± 0.37	± 0.48	± 0.45	± 0.49	± 0.54	± 0.52	± 0.54

+ Treatment means adjusted for differences in initial body weights.

No changes in organ weights or organ/body weight ratios were observed (Table 2). The haematology and clinical chemistry of the dogs remained unaffected throughout the exposure, the minor variations observed were not considered of any biological significance.

Table 2 - The organ weights and organ/body weight ratios of dogs fed Ionox 330 for two years

Dietary Concn (ppm)	Number of Animals	Terminal body wt (kg)	Organ weight (g)				
			Brain	Heart	Liver	Kidneys	Testes
Males							
0	5	14.8	83.5	126.1	451	68	16.2
2000	4	15.2	85.4	128.3	487	71	17.2
10000	4	14.9	84.6	118.6	410	67	17.9
S.E. of a treatment mean	5	± 0.61	± 3.28	± 6.76	± 32.5	± 3.2	± 1.05
	4	± 0.69	± 3.67	± 7.55	± 36.3	± 3.6	± 1.05
Females							
0	5	12.5	78.3	91.7	349	48	
2000	3	13.0	74.8	93.7	332	46	
10000	3	13.3	77.3	96.0	349	51	
S.E. of a treatment mean	5	± 0.42	± 2.62	± 4.73	± 25.2	± 4.2	
	3	± 0.54	± 3.38	± 6.11	± 32.6	± 5.4	
			Organ/body weight ratio (g/100 g body weight)				
Males							
0	5		0.57	0.85	3.02	0.46	0.11
2000	4		0.57	0.85	3.21	0.47	0.12
10000	4		0.57	0.80	2.75	0.45	0.12
S.E. of a treatment mean	5		± 0.033	± 0.044	± 0.145	± 0.018	± 0.011
	4		± 0.037	± 0.049	± 0.162	± 0.020	± 0.011
Females							
0	5		0.61	0.73	2.77	0.38	
2000	3		0.59	0.72	2.54	0.35	
10000	3		0.59	0.72	2.63	0.38	
S.E. of a treatment mean	5		± 0.013	± 0.028	± 0.135	± 0.021	
	3		± 0.016	± 0.036	± 0.174	± 0.027	

At autopsy, no gross or microscopic changes were observed. Summary of the pathological findings is shown in table 3. Lesions associated with ascarid infestation were detected in the livers of both treated and control animals. Evidence of renal and pulmonary disease was also found in all animals. Summary of the pathological findings is given in the tables below.

Table 3

a) Dogs killed during the experiment			
Dietary Concentration (ppm)	Animal Number	Time of Exposure (weeks)	Gross and Microscopic Pathological findings
2000	357	4	Focal meningitis, periarteritis of large intestine, haemorrhage of lymph nodes.
10000	360	12	Bronchopneumonia with numerous abscesses.

(Table 3 continues on the following page)

Table 3 (continued)

b) Dogs sacrificed after two years			
Dietary Concentration (ppm)	Animal Number	Sex	Gross and Microscopic Pathological findings
10000	340	Males	Hepatic granulomas, nephritis
	348		Negative
	351		Hepatic granulomas, nephritis
	352		Bronchopneumonia, pyelonephritis
	321	Females	Pneumonitis with granulomas, hepatic granulomas, nephritis
	343		Hepatic granulomas
	356		Hepatic granulomas, nephritis
2000	361	Males	Pulmonary fibrosis, parathyroid adenoma
	363		Focal pneumonitis
	339		Hepatic granulomas, nephritis
	354		Hepatic granulomas
	342	Females	Pneumonitis with granulomas, hepatic granulomas, pyelitis
	338		Nephritis and pyelitis
	345		Hepatic granulomas, renal abscess, resolving pneumonia
0	347	Males	Hepatic –peripertal inflammation
	349		Focal pulmonary fibrosis, fatty infiltration myocardium
	350		Interstitial pneumonitis with fibrosis, mild pyelitis
	353		Bronchitis
	355		Patchy pneumonitis, hepatic granulomas
	359	Females	Hepatic granulomas, nephritis
	341		Pneumonitis, hepatic granulomas, pyelitis
	344		Hepatic granulomas, nephritis
	346		Patchy pneumonitis, hepatic granulomas, nephritis
	358		Hepatic granulomas

In conclusion, this experiment showed no treatment-related toxicological effects to dogs for two years up to dose level of 10,000 ppm.

Remarks: This study was assigned a reliability code of 2e[2]
(Meets generally accepted scientific standards, well documented and acceptable for assessment).

Reference:

[1]Studies on the oral toxicity of Ionox 330: Two Year Experiment with Dogs. Shell Research Ltd. London, Tunstall Laboratories, Sittingbourne, UK. Project No. T507190/1. September 1968.

[2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

iii) Two-Year Oral Toxicity Study in Rats:

Test substance:	1,3,5-trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl)benzene CAS No. 1709-70-2
Method:	Groups of 25 males and 25 females, aged five weeks, individually caged, were given diets containing 400, 2000, and 10000 ppm of test material. Control groups, 45 males and 45 females were given the powdered diet, Diet 86 powder. Additional rats, 15 males and 15 females, were fed the control and treated diets and 5 of each sex, from each treatment, were killed and autopsied after 26, 52, and 78 weeks feeding. The animals were allocated at random to the treatment groups. Daily observations were made on the general health and behavior of all animals. Their body weights and food intakes were recorded weekly for the first 13 weeks of the experiment and thereafter at 4 week intervals. At autopsy, a gross pathological examination was made and the major visceral organs weighed. Histopathological examination was made on haematoxylin and eosin stained sections of liver, thyroid, parathyroid, heart, lungs, spleen, kidneys, adrenals, small and large intestines, stomach, pancreas, skin, skeletal muscle, salivary glands, lymph nodes, bladder, testes, prostate, uterus, ovaries, fallopian tubes, eye, and brain of all animals. Statistical analysis was done on body and organ weights.[1]
Species/strain:	Carworth farm E Strain rats
Initial age of the animals:	5 weeks old
Total number of rats:	360 rats:
No. of animals per group:	40 males and 40 females - test group 60 males and 60 females - control group
Route of administration:	Dietary
Exposure period:	2 years
Dose:	0, 400, 2000, and 10000 ppm
GLP:	No
Year:	1968
Results:	Throughout the two-year experiment, the general health, behavior and mortality of the treated and control group of rats were similar. There were no mortalities. The body weights and food intakes of the treated groups with the exception of the 10,000 ppm females were unaffected. The

body weights of the 10000 ppm females were reduced, particularly during the second year of exposure. This body weight loss may be due to lower initial body weight of females surviving for two years. (see the tables below). The reduction in food intake of these females occurred throughout the experiment. No adverse effects were seen related to food consumption, behavioral reactions, urine analysis, gross pathologic studies, and histopathologic studies. Hematologic and clinical studies revealed no significant differences between treated and untreated animals.

After two years, the liver and kidney weights and liver/body weight ratios of the 10,000 ppm females were reduced (Table 1 and 2). These changes were possibly related to the initial reduced body weights of this group. The organ weights and organ/body weight ratios of the treated animals were unaffected at the 6, 12, and 18 month exposure intervals (Table 3).

Table 1. Organ weights after 2 years exposure - males

Organ weights data for male rats (in grams)

Dietary Level (ppm)	No. of rats	Body weight (g)		Organ weight (g)				
		Initial	Terminal	Brain	Heart	Liver	Kidneys	Testes
0	21	90	388	1.96	1.57	15.86	3.22	3.53
400	9	86	391	1.93	1.56	15.50	3.21	3.62
2000	15	92	391	1.97	1.51	15.25	3.11	3.48
10000	10	78	399	1.94	1.46	15.46	3.21	3.85

Organ/body weight ratios for male rats (in grams)

Dietary Level (ppm)	No. of rats	Body weight (g)		Organ/ body weight ratio (g/100 g body weight)				
		Initial	Terminal	Brain	Heart	Liver	Kidneys	Testes
0	21	90	388	0.51	0.41	4.08	0.83	0.91
400	9	86	391	0.50	0.40	3.96	0.83	0.92
2000	15	92	391	0.51	0.39	3.91	0.80	0.89
10000	10	78	399	0.49	0.37	3.88	0.81	0.96

Table 2. Organ weights after 2 years exposure - females

Organ weights data for female rats (in grams)

Dietary Level (ppm)	No. of rats	Body weight (g)		Organ weight (g)			
		Initial	Terminal	Brain	Heart	Liver	Kidneys
0	26	103	334	1.86	1.27	14.28	2.61
400	15	105	333	1.85	1.36	14.37	2.75
2000	13	96	332	1.82	1.35	14.26	2.52
10000	14	91*	317	1.83	1.20	12.47**	2.35**

Organ/body weight ratios for female rats (in grams)

Dietary Level (ppm)	No. of rats	Body weight (g)		Organ/ body weight ratio (g/100 g body weight)			
		Initial	Terminal	Brain	Heart	Liver	Kidneys
0	21	90	388	0.56	0.37	4.30	0.79
400	9	86	391	0.56	0.42	4.33	0.84
2000	15	92	391	0.55	0.41	4.32	0.75
10000	10	78	399	0.58	0.37	3.93*	0.76

*P <0.05)
**P <0.01) Significance of difference between treatment and control means

Table 3

Organ weights of rats fed with test substance

After 26 weeks (Males)

Dietary Level (ppm)	No. of rats	Body weight (g)	Organ weight (g)				
		Terminal	Brain	Heart	Liver	Kidneys	Testes+
0	5	433	1.77	1.33	14.31	2.80	3.77
400	5	447	1.93	1.27	16.41	3.07	3.61
2000	5	425	1.91	1.25	16.09	2.92	3.85
10000	5	423	1.92	1.14	16.10	3.01	3.60
Standard error of treatment Mean		± 12.5	± 0.08	± 0.09	± 0.90	± 0.14	± 0.11

After 26 weeks (Females)

Dietary Level (ppm)	No. of rats	Body weight (g)	Organ weight (g)			
		Terminal	Brain	Heart	Liver	Kidneys
0	5	289	1.86	0.90	9.80	1.96
400	5	297	1.82	0.93	10.46	1.95
2000	5	297	1.83	0.92	9.65	1.92
10000	5	292	1.84	0.92	9.84	1.89
Standard error of treatment Mean		± 6.4	± 0.02	± 0.03	± 0.48	± 0.08

+ Treatment means adjusted for differences in the initial body weight.

Table 3 (continued)

Organ weights of rats fed with test substance

After 52 weeks (Males)

Dietary Level (ppm)	No. of rats	Body weight (g)	Organ weight (g)				
		Terminal	Brain	Heart	Liver	Kidneys	Testes+
0	4	478	1.99	1.33	17.13	3.32	3.98
400	5	453	2.00	1.34	17.15	3.28	3.69
2000	5	469	1.92	1.35	18.35	3.46	3.94
10000	5	437*	1.96	1.38	17.54	3.13	3.58
Standard error of treatment Mean	4 5	± 14.1 ± 12.6	± 0.029 ± 0.026	± 0.052 ± 0.047	± 1.303 ± 1.166	± 0.119 ± 0.106	± 0.283 ± 0.245

After 52 weeks (Females)

Dietary Level (ppm)	No. of rats	Body weight (g)	Organ weight (g)			
		Terminal	Brain	Heart	Liver	Kidneys
0	5	330	1.83	1.06	12.17	2.37
400	5	322	1.86	1.08	11.54	2.17
2000	5	326	1.82	1.05	12.29	2.50
10000	5	297	1.80	0.97	10.60	2.10
Standard error of treatment Mean	5	± 13.3	± 0.046	± 0.048	± 0.614	± 0.123

+ Treatment means adjusted for differences in the initial body weight.
* Significance of difference between treatment and control means

Table 3 (continued)

Organ weights of rats fed with test substance

After 78 weeks (Males)

Dietary Level (ppm)	No. of rats	Body weight (g) Terminal	Organ weight (g) Brain	Heart	Liver	Kidneys	Testes+
0	5	461	1.92	1.40	16.35	3.48	4.78
400	5	455	1.96	1.35	16.52	3.29	3.68
2000	5	439	1.96	1.30	16.21	3.32	4.10
10000	4	441	1.95	1.31	15.45	3.41	3.80
Standard error of treatment Mean	5 4	± 5.56 ± 6.3	± 0.036 ± 0.040	± 0.031 ±0.035	± 1.164 ± 1.301	± 0.209 ± 0.230	± 0.543 ± 0.607

After 78 weeks (Females)

Dietary Level (ppm)	No. of rats	Body weight (g) Terminal	Organ weight (g) Brain	Heart	Liver	Kidneys
0	5	295	1.85	1.06	11.03	3.10
400	4	306	1.84	1.09	11.87	2.48
2000	4	289	1.79	1.03	10.25	2.19
10000	4	317	1.80	1.09	11.76	2.64
Standard error of treatment Mean	5 4	± 10.3 ± 11.5	± 0.030 ± 0.034	± 0.048 ± 0.054	± 0.712 ± 0.796	± 0.444 ± 0.496

+ Treatment means adjusted for differences in the initial body weight.

No gross or microscopic lesions attributable to the exposure were found. The most commonly occurring lesions in the renal and cardio-vascular systems are typical of the senile changes which occur in ageing rats. The incidence of these lesions was greater in the animals surviving two years, than in those dying during the experiment. The tumor incidence was similar among the treated and control animals. Thyroid tumors predominated in the animals, of both sexes, killed after 2 years exposure while mammary tumors occurred throughout the experiment.

The lack of any pathological or other change indicating a toxic effect of test substance suggests that the slight changes in body and organ weights of the 10,000 ppm females were probably partly related to the smaller body weights of the surviving animals.

Remarks:	This study was assigned a reliability code of 2e[2] (Meets generally accepted scientific standards, well documented and acceptable for assessment).
Reference:	[1]Studies on the oral toxicity of Ionox 330, Two year experiment with rats. TLGR. 0023.68, Project No. T507190/1, November 1968. Tunstall laboratory. [2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

18. REPRODUCTIVE / DEVELOPMENTAL TOXICITY

Test substance:	1,3,5-trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl)benzene CAS No. 1709-70-2 Batch No: 89/5/6232, purity 98%
Method:	In this study, 80 rats were used. The animals were bred and maintained under specific-pathogen-free conditions. Groups of 20 males and 20 female rats aged three weeks were caged in groups of two per each sex. Diet containing 5000 ppm Ionox 330 was received. Control groups received untreated diet. The animals were allocated at random to the treatment groups and caged together for mating. The pair remained together throughout the experiment and three litters were bred. Daily observations were made on the general health and behavior of all animals. Records were kept of dates of mating, intervals between mating and number of pregnancies. The number of young in each litter were counted at birth, after five days and at weaning (21 days). The adult animals were autopsied after the third litter and 10 males and 10 females from F3c, third generation were examined for teratogenic effects. A wide range of organs and tissues were examined histologically. An outline of a three generation reproduction study in rats is shown in Figure 1. Statistical analyses of body and organ weights were made using the initial body weight as a covariate in covariance analysis.[1]
Species/strain:	Rats: Carworth Farm E strain
Age:	3 weeks old
No. of animals:	80 rats in total 20 males and 20 females / group
Dose:	0, 5000 ppm
GLP:	No
Year:	1970
Result:	The general health and behavior of the treated and control groups were similar. No deaths occurred. No effects on reproduction, number of pregnancies, litters and young born were observed in rats fed with Ionox 330 from weaning to maturity for three generations (Table 1). No lesions were detected. No gross or histological changes were noted. In conclusion, rats fed with 5000 ppm for three generations produced no effects upon reproduction.

Table 1

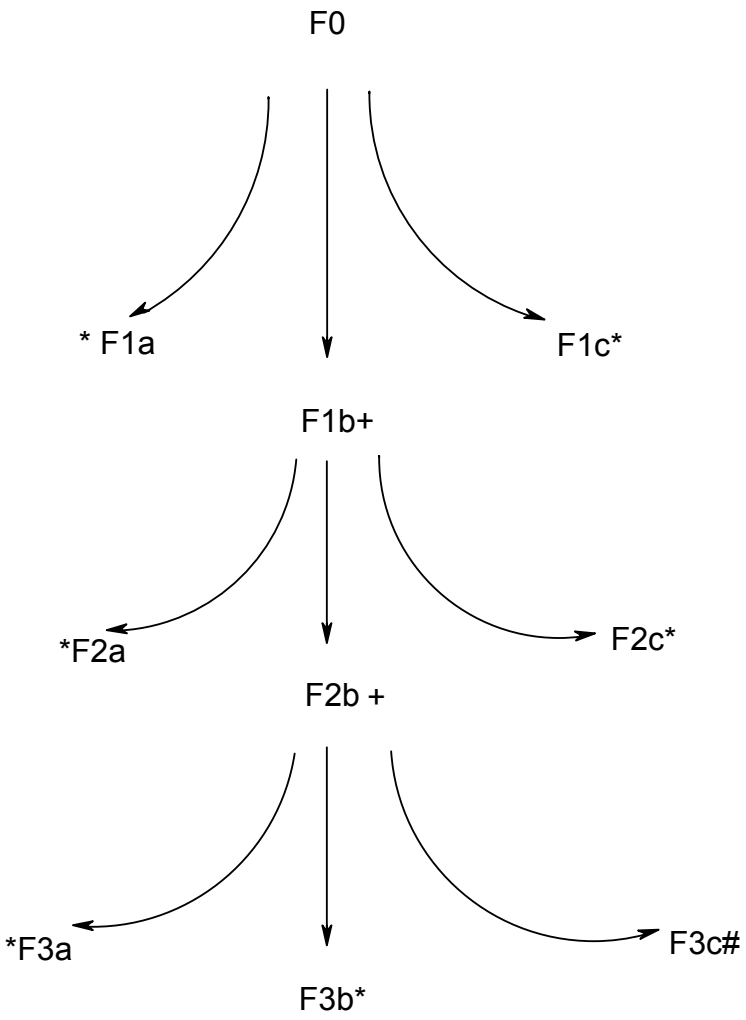
Reproductive Performance of Rats

Generation/ Litter	Dietary Conc. (ppm)	No. of pregnancies	No. of young born		No. of young alive		No. of young alive after 21 days	Fertility Index [a]	Gestation Index [b]	Viability Index [c]	Lactation Index [d]
Generation 1											
	0	19/20	220	(11.6) +	218	(11.5) +	181	95	100	99.1	83.0
Litter F1a	5000	19/20	221	(11.6)	218	(11.5)	158	95	100	98.6	72.5
Litter F1b	0	19/20	178	(9.4)	178	(9.4)	140	95	100	100	78.7
	5000	19/20	171	(9.0)	171	(9.0)	130	95	100	100	76.0
Litter F1c	0	19/20	187	(9.8)	187	(9.8)	160	95	100	100	85.6
	5000	19/20	192	(10.1)	192	(10.1)	167	95	100	100	87.0
Generation 2											
	0	20/20	236	(11.8)	236	(11.8)	173	100	100	100	73.3
Litter F2a	5000	20/20	223	(11.2)	223	(11.2)	207	100	100	100	92.8
Litter F2b	0	20/20	190	(9.5)	190	(9.5)	175	100	100	100	92.1
	5000	20/20	190	(9.5)	190	(9.5)	183	100	100	100	96.3
Litter F2c	0	20/20	209	10.5)	209	(10.5)	189	100	100	100	90.4
	5000	*19/20	169	(8.9)	169	(8.9)	157	95	100	100	92.9
Generation 3											
	0	19/20	219	(11.5)	219	(11.5)	204	95	100	100	93.2
Litter F3a	5000	20/20	232	(11.6)	232	(11.6)	217	100	100	100	93.5
Litter F3b	0	18/20	189	(10.5)	189	(10.5)	181	90	100	100	95.8
	5000	20/20	189	(9.5)	189	(9.5)	172	100	100	100	91.0
Litter F3c	0	18/20	178	(9.9)	178	(9.9)	165	90	100	100	92.7
	5000	20/20	207	(10.4)	207	(10.4)	191	100	100	100	92.3

* Female sent to pathology
\+ Average number of pups/ litter
(a) Fertility Index - % matings resulting in pregnancies
(b) Gestation Index - % pregnancies resulting in birth of live litters
(c) Viability Index - % young born alive after 2 days
(d) Lactation Index - % young alive after 2 days surviving to weaning at 21 days.

Figure 1

An Outline of a three generation reproduction study in rats



* Litters killed at weaning
\+ Litters used for selection of breeding animals
\# Litter used for teratogenic examination

Remarks: This study was assigned a reliability code of 2e[2] (Meets generally accepted scientific standards, well documented and acceptable for assessment).

Reference:

[1]Studies on the oral toxicity of Ionox 330: Three-Generation Reproduction Study in Rats. Shell Research Ltd. London, Tunstall Laboratories, Sittingbourne, UK. Project No. T507190/1. February 1970.

[2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

19. GENERAL REFERENCE

Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

Definition of codes

1 = Valid without restriction
1a: GLP guideline study
1b: Comparable to guideline study
1c: Meets national standard methods (AFNOR/DIN)
1d: Meets generally accepted scientific standards and is described in sufficient detail

2 = Valid with restriction
2a: Guideline study without detailed documentation
2b: Guideline study with acceptable restrictions
2c: Comparable to guideline study with acceptable restrictions
2d: Meets national standard methods with acceptable restrictions
2e: Meets generally accepted scientific standards, well documented and acceptable for assessment
2f: Accepted calculation method
2g: Data from Handbook or collection of data

3 = Invalid
3a: Documentation insufficient for assessment
3b: Significant methodological deficiencies
3c: Unsuitable test system

4 = Not assignable
4a: Abstract
4b: Secondary literature
4c: Original reference not yet available
4d: Original reference in foreign language
4e: Documentation in sufficient for assessment