

# Robust Summaries and Dossier for CAS No. 40601-76-1

**Existing Chemical** : ID: 40601-76-1  
**CAS No.** : 40601-76-1  
**EINECS Name** : 1,3,5-Tris[[4-(1,1-dimethylethyl)-3-hydroxy-2,6-xylyl]methyl]1,3,5-triazine-2,4,6(1H,3H,5H)-trione

## Producer related part

**Company** : Cytec Industries Inc.  
**Creation date** : 15.05.2003

## Substance related part

**Company** : Cytec Industries Inc.  
**Creation date** : 15.05.2003

**Status** :  
**Memo** :

**Printing date** : 09.09.2003  
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**Chapter (profile)** : Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10  
**Reliability (profile)** : Reliability: without reliability, 1, 2, 3, 4  
**Flags (profile)** : Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

# 1. General Information

Id 40601-76-1  
Date 26.05.2003

## 1.0.1 APPLICANT AND COMPANY INFORMATION

## 1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

## 1.0.3 IDENTITY OF RECIPIENTS

## 1.0.4 DETAILS ON CATEGORY/TEMPLATE

### 1.1.0 SUBSTANCE IDENTIFICATION

**IUPAC Name** : None assigned  
**Smiles Code** : O=C3(N(Cc1(c(C)c(O)c(C(C)(C)C)cc1C))C(=O)N(Cc2(c(C)c(O)c(C(C)(C)C)cc2C))C(=O)N3Cc4(c(C)c(O)c(C(C)(C)C)cc4C))  
**Molecular formula** : C42 H57 N3 O6  
**Molecular weight** : 699.94  
**Petrol class** :

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### 1.1.1 GENERAL SUBSTANCE INFORMATION

**Purity type** :  
**Substance type** : organic  
**Physical status** : solid  
**Purity** : = 93.7 - 98.9 g/kg  
**Colour** : off-white powder  
**Odour** : none

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### 1.1.2 SPECTRA

## 1.2 SYNONYMS AND TRADENAMES

1,3,5-triazine-2,4,6(1H,3H,5H)-trione

1,3,5-tris[[4-tert-butyl-3-hydroxy-2,6-xylyl]methyl]-1,3,5-triazine-2,4,6(1H,3H,5H)-trione

1,3,5-tris(2,6-dimethyl-3-hydroxy-4-tert-butylbenzyl) isocyanurate

1,3,5-tris[[4-(1,1-dimethylethyl)-3-hydroxy-2,6-dimethylphenyl]methyl]-

1,3,5-Tris(4-tert-butyl-3-hydroxy-2,6-dimethylbenzyl)-1,3,5-triazine-2,4,6(1H,3H,5H)-trione

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1,3,5-Tris(4-tert-butyl-3-hydroxy-2,6-dimethylbenzyl)-s-triazine-2,4,6(1H,3H,5H)-trione

Cyanox (TM) 1790 Antioxidant

Cyanox 1790

Cyanox 1970

Cynomix 1790

Triazine(s)-2,4,6-(1H,3H,5H)trione

tris(4-t-butyl-3-hydroxy-2,6-dimethyl-benzyl)-

Tris(4-tert-butyl-2,6-dimethyl-3-hydroxybenzyl)isocyanurate

Tris(4-tert-butyl-3-hydroxy-2,6-dimethylbenzyl)isocyanurate

## 1.3 IMPURITIES

## 1.4 ADDITIVES

## 1.5 TOTAL QUANTITY

### 1.6.1 LABELLING

### 1.6.2 CLASSIFICATION

### 1.6.3 PACKAGING

## 1.7 USE PATTERN

### 1.7.1 DETAILED USE PATTERN

### 1.7.2 METHODS OF MANUFACTURE

**1.8 REGULATORY MEASURES**

**1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES**

**1.8.2 ACCEPTABLE RESIDUES LEVELS**

**1.8.3 WATER POLLUTION**

**1.8.4 MAJOR ACCIDENT HAZARDS**

**1.8.5 AIR POLLUTION**

**1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES**

**1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS**

**1.9.2 COMPONENTS**

**1.10 SOURCE OF EXPOSURE**

**1.11 ADDITIONAL REMARKS**

**1.12 LAST LITERATURE SEARCH**

**1.13 REVIEWS**

**2.1 MELTING POINT**

<b>Value</b>	:	= 159 - 162 °C	
<b>Sublimation</b>	:		
<b>Method</b>	:	other	
<b>Year</b>	:		
<b>GLP</b>	:	no data	
<b>Test substance</b>	:	as prescribed by 1.1 - 1.4	
<b>Reliability</b>	:	(2) valid with restrictions Experimental details were not provided.	
<b>Flag</b>	:	Critical study for SIDS endpoint	
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<b>Value</b>	:	ca. 349.8 °C	
<b>Remark</b>	:	Value given is the average of the Joback, Gold and Ogle methods.	
<b>Reliability</b>	:	(2) valid with restrictions Data were obtained by EPIWIN modeling.	
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**2.2 BOILING POINT**

<b>Value</b>	:	ca. 926 °C at 1013 hPa	
<b>Decomposition</b>	:		
<b>Method</b>	:	other	
<b>Year</b>	:	2003	
<b>GLP</b>	:	no	
<b>Test substance</b>	:	as prescribed by 1.1 - 1.4	
<b>Reliability</b>	:	(2) valid with restrictions Data were obtained by EPIWIN modeling.	
<b>Flag</b>	:	Critical study for SIDS endpoint	
19.05.2003			(11)

**2.3 DENSITY****2.3.1 GRANULOMETRY****2.4 VAPOUR PRESSURE**

<b>Value</b>	:	ca. 000013 hPa at 25 °C	
<b>Decomposition</b>	:		
<b>Method</b>	:	other (calculated)	
<b>Year</b>	:	2003	
<b>GLP</b>	:	no	
<b>Test substance</b>	:	as prescribed by 1.1 - 1.4	
<b>Remark</b>	:	Inputs to the program are the CAS No. 40601-76-1 and a measured melting point of 160.5 degrees C.	
<b>Reliability</b>	:	(2) valid with restrictions Data were obtained by EPIWIN modeling.	
<b>Flag</b>	:	Critical study for SIDS endpoint	

## 2. Physico-Chemical Data

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### 2.5 PARTITION COEFFICIENT

**Partition coefficient** : octanol-water  
**Log pow** : = 15.281 at °C  
**pH value** :  
**Method** : other (calculated)  
**Year** : 2003  
**GLP** : no  
**Test substance** : as prescribed by 1.1 - 1.4

**Remark** : Inputs to the program are the CAS No. 40601-76-1 and a measured melting point of 160.5 degrees C.

**Reliability** : (2) valid with restrictions  
Data were obtained by EPIWIN modeling.

**Flag** : Critical study for SIDS endpoint

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### 2.6.1 SOLUBILITY IN DIFFERENT MEDIA

**Solubility in Value** : Water  
: = 2.425 E-11 mg/l at 25 °C  
**pH value concentration** : at °C  
**Temperature effects** :  
**Examine different pol.** :  
**pKa** : at 25 °C  
**Description** :  
**Stable** :

**Remark** : Inputs to the program are the CAS No. 40601-76-1 and a measured melting point of 160.5 degrees C.

**Reliability** : (2) valid with restrictions  
Data were obtained by EPIWIN modeling.

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**Solubility in Value** : Water  
: at °C  
**pH value concentration** : at °C  
**Temperature effects** :  
**Examine different pol.** :  
**pKa** : at 25 °C  
**Description** :  
**Stable** :

**Remark** : Negligible solubility in water.

**Reliability** : (4) not assignable  
Experimental details were not provided.

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### 2.6.2 SURFACE TENSION

## 2. Physico-Chemical Data

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2.7 FLASH POINT

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

### 3. Environmental Fate and Pathways

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#### 3.1.1 PHOTODEGRADATION

Type : air  
Light source : Sun light  
Light spectrum : nm  
Relative intensity : based on intensity of sunlight

##### INDIRECT PHOTOLYSIS

Sensitizer : OH  
Conc. of sensitizer :  
Rate constant : = .000000000908947 cm<sup>3</sup>/(molecule\*sec)  
Degradation : = 50 % after 1.4 hour(s)  
Deg. product :  
Method : other (calculated)  
Year : 2003  
GLP : no  
Test substance : as prescribed by 1.1 - 1.4

Remark : Inputs to the program are the CAS No. 40601-76-1 and a measured melting point of 160.5 degrees C.  
Atmospheric photodegradation is not expected to be a significant route of elimination, since the substance has very limited volatility.

Reliability : (2) valid with restrictions  
Data were obtained by EPIWIN modeling.

Flag : Critical study for SIDS endpoint  
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#### 3.1.2 STABILITY IN WATER

Type :  
t1/2 pH4 : at °C  
t1/2 pH7 : ca. 1 year at 20 °C  
t1/2 pH9 : at °C  
Deg. product :  
Method : other (calculated)  
Year : 2003  
GLP : no  
Test substance : as prescribed by 1.1 - 1.4

Remark : Inputs to the program are the CAS No. 40601-76-1 and a measured melting point of 160.5 degrees C.  
EPIWIN Hydrowin estimates that the rate of hydrolysis in neutral water at ambient temperatures will be extremely slow, with a half life of > 1 year. This estimate is based on the presence of urea functional groups in the molecule.

Reliability : (2) valid with restrictions  
Data were obtained by EPIWIN modeling.

Flag : Critical study for SIDS endpoint  
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#### 3.1.3 STABILITY IN SOIL

#### 3.2.1 MONITORING DATA

## 3.2.2 FIELD STUDIES

## 3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

<b>Type</b>	: fugacity model level III
<b>Media</b>	: soil - air
<b>Air</b>	: 0 % (Fugacity Model Level III)
<b>Water</b>	: 1.28 % (Fugacity Model Level III)
<b>Soil</b>	: % (Fugacity Model Level I)
<b>Biota</b>	: 67.1 % (Fugacity Model Level II/III)
<b>Soil</b>	: 31.6 % (Fugacity Model Level II/III)
<b>Method</b>	: other
<b>Year</b>	: 2003
<b>Remark</b>	: Inputs to the program are the CAS No. 40601-76-1 and a measured melting point of 160.5 degrees C. Emission rates are assumed to be 0 kg/hr to air, 1000 kg/hr to water, 1000 kg/hr to soil and 1000 kg/hr to sediment.
<b>Result</b>	: Half-lives in various media are air: 2.825 hours; water; 3600 hours; soil: 3600 hours; and sediment: 1.44 E+4 hours. The Henry's Law Constant [calculated by EPIWIN Henry (v3.10)] is 1.7E-27 atm-m <sup>3</sup> /mol (bond est.). The soil-sediment coefficient Koc [calculated by EPIWIN PCKOC (v1.66)] is Koc = 1E+10.
<b>Reliability</b>	: (2) valid with restrictions Data were obtained by EPIWIN modeling.
<b>Flag</b>	: Critical study for SIDS endpoint
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## 3.3.2 DISTRIBUTION

## 3.4 MODE OF DEGRADATION IN ACTUAL USE

## 3.5 BIODEGRADATION

<b>Type</b>	: aerobic
<b>Inoculum</b>	: other bacteria: secondary effluent from wastewater treatment plant
<b>Contact time</b>	: 28 day(s)
<b>Degradation</b>	: = 25.9 (±) % after 28 day(s)
<b>Result</b>	: other: not readily biodegradable
<b>Kinetic of testsubst.</b>	: 5 day(s) = 2.3 % 10 day(s) = 5.4 % 14 day(s) = 9.3 % 20 day(s) = 17.2 % 28 day(s) = 24.7 %
<b>Control substance</b>	: Benzoic acid, sodium salt
<b>Kinetic</b>	: % %
<b>Deg. product</b>	: not measured
<b>Method</b>	: OECD Test Guideline 301 B "Ready Biodegradability: Modified Sturm Test (CO <sub>2</sub> evolution)"
<b>Year</b>	: 2002
<b>GLP</b>	: yes
<b>Test substance</b>	: as prescribed by 1.1 - 1.4

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- Remark** : The authors stated that "the test substance was not toxic to the inoculum since biodegradation occurred in a mixed solution containing test and reference materials". However, it is clear that the test material was toxic to the inoculum, since the amount of biodegradation occurring in the flask containing both the reference and test material was less than that of the reference material alone.
- Result** : The cumulative biodegradation rates (in %) for the two concentrations of test material at 3, 5, 7, 10, 14, 17, 20, 24, 28 and 29 days were 1.0 and 1.0, 2.1 and 2.4, 4.5 and 4.57, 4.98 and 5.83, 8.28 and 10.4, 13.3 and 15.4, 16.3 and 18.1, 17.4 and 21.8, 21.7 and 27.6 and 23.2 and 28.5, respectively. At the same time intervals, values (in %) for inoculum controls were 12.9 and 12.7, 15.1 and 15.4, 17.2 and 14.4, 22.0 and 22.7, 25.6 and 23.5, 20.7 and 21.2, 24.4 and 23.4, 25.5 and 24.5, 23.3 and 23.4 and 20.1 and 19.6, respectively. The cumulative biodegradation rate for the reference material at 3, 5, 7, 10, 14, 17, 20, 24, 28 and 29 days was 19.0, 37.0, 54.0, 62.4, 68.1, 71.6, 73.6, 74.4, 76.4 and 77.1, respectively. The cumulative biodegradation rate for the reference plus test material at 3, 5, 7, 10, 14, 17, 20, 24, 28 and 29 days was 9.5, 18.4, 26.9, 32.0, 35.4, 37.3, 38.3, 38.8, 39.6 and 40.5, respectively.

Since a  $\geq 60\%$  biodegradation rate was not achieved within 28 days, the material was not deemed readily biodegradable. The test was valid, since the total amount of CO<sub>2</sub> evolution in the inoculum blank at the end of the test was within the acceptable range for a valid test, and the cumulative biodegradation of the reference material (68.1% on day 14) was  $\geq 60\%$  by day 14. The difference in CO<sub>2</sub> evolution between extreme duplicate values of test material at the end of the test (18.6%) also met the requirements of the guideline ( $< 20\%$ ).

- Test condition** : Water used for the preparation of test media was collected from a water purification system using both ion exchange and carbon filtration treatment. The test water had a total organic carbon (TOC) content  $< 1$  mg/l. TOC analysis was done using a DC-80 TOC analyzer system. This water was used to make a defined mineral medium containing phosphate buffer, ferric chloride (final concentration 0.25 mg/l), magnesium sulfate (final concentration 22.5 mg/l) and calcium chloride (final concentration 27.5 mg/l). The test material was not soluble enough in water to make a stock solution, so the material was weighed into weigh boats and added dry to test bottles. The weigh boats were washed with mineral medium to remove as much material from them as possible. The carbon content of the test material was analyzed with an elemental analyzer. It contained the equivalent carbon concentration of 71.9%. A stock solution of the reference material (1000 mg ai/l 99.6% pure sodium benzoate) was prepared on the day of the test which had an equivalent carbon concentration of 58.3%.

A total of six 4-liter bottles were prepared. On day minus one, 100 ml of inoculum and 2400 ml of mineral medium were added to each flask. The inoculum was the glass wool filtrate from 2 liters of secondary effluent collected on May 30, 2002 from the Loxahatchee River Environmental Control District Wastewater Treatment Plant in Jupiter, Florida. The contents of each bottle were purged with CO<sub>2</sub>-free air for 24 hours prior to test initiation. The airflow was controlled by flowmeters and regulated to 40 mg/l for each bottle. Check valves were inserted in the lines between the gas washing bottles and the test bottles.

After the bottles were purged with CO<sub>2</sub>-free air, 500 ml of mineral medium was added to two bottles that served as inoculum blanks (no test material). Sodium benzoate (103 ml of stock solution, for a concentration of 20.0 mg carbon/l) and 397 ml of mineral medium was added to another bottle. Two bottles containing test material were prepared (one containing 0.0836 g of test material and 500 ml of mineral medium, for a concentration of 20.1 mg carbon/l and another containing 0.0846 g of test material and 500 ml of

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mineral medium, for a concentration of 20.3 mg carbon/l). The final bottle received 0.0836 g test material, 103 ml of the sodium benzoate stock solution and 397 ml of the mineral medium (20.1 mg carbon/l test material and 20.0 mg carbon/l reference material, for a total of 40.1 mg carbon/l). Each bottle was capped with a silicone stopper. Inlet and outlet tubing was built into each stopper. All of the bottles were placed in an environmental chamber maintained at 22 +/- 2 degrees C, and aerated through the inlet tubing. The medium in each bottle was continuously stirred with a magnetic stirrer.

The evolved CO<sub>2</sub> gas that came out of each bottle was trapped in a series of three gas-washing bottles containing 100 ml of 0.0125 M Ba(OH)<sub>2</sub>. The amount of unreacted Ba(OH)<sub>2</sub> was quantified by titrating with a HCl solution to the phenolphthalein endpoint. During the first week of the study, CO<sub>2</sub> was analyzed every 2-3 days. After the first week, the sampling schedule was changed to once every 3-5 days. The test was terminated on day 28. On day 28, aeration was stopped, the lids on the bottles were opened and 1 ml of concentrated HCl was added to each bottle to release any CO<sub>2</sub> remaining in the test vessel. Final titrations were made on day 29.

The percent biodegradability at each time interval was calculated for each test condition using the following equation: percent biodegradability = cumulative CO<sub>2</sub> produced (mg)/ThCO<sub>2</sub> (mg) x 100, where ThCO<sub>2</sub> (mg) = carbon added (mg) x 44 mg as CO<sub>2</sub>/12 mg as carbon. The amount of CO<sub>2</sub> produced at each time interval = K(50-Vt), where K = 1.10 (the constant which converts volume of HCl titrated to mg CO<sub>2</sub> produced), 50 = volume (ml) needed to titrate 100 ml Ba(OH)<sub>2</sub> and Vt = volume (ml) HCl titrated into the test solution. The amount of CO<sub>2</sub> produced in the inoculum only control was subtracted from that produced by the test or reference materials to obtain the amount of CO<sub>2</sub> produced from these materials.

- Test substance** : The test material was Cyanox 1790, used as supplied by the manufacturer. According to the MSDS, the purity of this material is 93.7-98.9%. Impurities are not listed on the MSDS.
- Reliability** : (2) valid with restrictions  
The concentration of material tested was toxic to the inoculum. The actual concentration used was not confirmed analytically, and the material is insoluble in water at the concentrations tested.
- Flag** : Critical study for SIDS endpoint  
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#### 3.6 BOD<sub>5</sub>, COD OR BOD<sub>5</sub>/COD RATIO

#### 3.7 BIOACCUMULATION

- Species** : other  
**Exposure period** : at °C  
**Concentration** :  
**Elimination** :  
**Method** : other  
**Year** : 2003  
**GLP** : no  
**Test substance** : as prescribed by 1.1 - 1.4

- Remark** : Inputs to the program are the CAS No. 40601-76-1 and a measured melting point of 160.5 degrees C. The estimate is based on the presence of

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**Result** : three phenol groups on the molecule.  
: The log of bioconcentration factor [calculated by EPIWIN BCF (v2.14)] is  
log BCF = 0.500. The BCF is 3.162.

**Reliability** : (2) valid with restrictions  
Data were obtained by EPIWIN modeling.

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#### 3.8 ADDITIONAL REMARKS

**4.1 ACUTE/PROLONGED TOXICITY TO FISH**

**Type** : static  
**Species** : Oncorhynchus mykiss (Fish, fresh water)  
**Exposure period** : 96 hour(s)  
**Unit** : mg/l  
**NOEC** : = 100 measured/nominal  
**LC50** : > 100 measured/nominal  
**Limit test** : yes  
**Analytical monitoring** : no  
**Method** : OECD Test Guideline 203 "Fish, Acute Toxicity Test"  
**Year** : 2002  
**GLP** : yes  
**Test substance** : as prescribed by 1.1 - 1.4

**Result** : None of the control or treated fish died or exhibited signs of toxicity. The No effect concentration (NOEC) and 96-hour LC50 values are therefore > 100 mg whole material/l.

**Test condition**

The temperature ranged from 14.2 - 14.6 degrees C during the test. The dissolved oxygen concentrations in the test water ranged from 10.4 to 10.6 mg/l (102-104 % of saturation) at the beginning of the test and 8.4 to 8.8 mg/l (82-86% of saturation) for the remainder of the test. pH values ranged from 7.4 to 7.6 at the beginning of the test and were all 7.0 at the end of the test.

: Juvenile rainbow trout (4.0 +/- 0.23 cm avg length and 0.8569 +/- 0.12 g average wet weight) were acclimated for 12 days before treatment. They were maintained in filtered laboratory freshwater at a temperature of 13.0 to 15.0 degrees C. They were fed a diet of salmon starter up to 24 hours before treatment. Mortality during the 7-day period prior to testing was 0%.

The dilution water was a moderately hard freshwater, which originated from Jupiter Florida. It was treated by vigorous aeration, filtered to 20 microns, passed through activated carbon and re-aerated prior to use. The hardness (as calcium carbonate), alkalinity (as calcium carbonate) and specific conductivity were 90 mg/l, 31 mg/l and 377 microSiemens, respectively. Chemical characteristics were listed in an Appendix to the study.

A preliminary 96-hr range-finding test was conducted at nominal concentrations of 0.0 (control), 0.01, 0.10, 1.05, 10.0 and 100 mg whole material/l. Five fish were tested for each condition. None of the fish died in the experiment, and the material was insoluble at each concentration used.

Due to the insolubility of the test material in the preliminary test, the definitive test was initiated as a limit test with 100 mg whole material/l. Each of three replicates was spiked by direct addition of the test material (0.9 g) into 9 liters of dilution water. The replicate test chambers were placed onto mechanical stir plates with rotating stir bars. The test material was spiked directly into the vortex and allowed to mix for approximately 25 minutes. The liquid in each of the test chambers was then filtered through a Gelman minicapsule (0.45 microns) Versapore membrane filter. The test filtrate (which appeared clear) was transferred into glass test chambers, placed onto a 15 degrees C water table, and allowed to reach the desired test temperature (15 +/- 1 degrees C). Concurrent controls also were prepared in triplicate.

After initial water quality parameters (temperature, pH, dissolved oxygen) were measured, 7 fish (one or two at a time) were added to each test

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	chamber (loading was 0.67 g fish/l). The test chambers were 10 liter glass tanks (43 cm x 23 cm x 14 cm) containing 9 liters of dilution water and providing a final water depth of 10 cm. All chambers were covered to reduce evaporation. The chambers were placed in a water bath and maintained under a photoperiod of 16 hours light/8 hours darkness (at 6-7 micromoles/m <sup>2</sup> /sec light intensity). Fish were not fed and water was not aerated during the test. Survival of fish and water quality were monitored daily. The test was terminated after 96-hr evaluations were performed.
<b>Test substance</b>	: The test material was Cyanox 1790, used as supplied by the manufacturer. According to the MSDS, the purity of this material is 93.7-98.9%. Impurities are not listed on the MSDS.
<b>Reliability</b>	: (2) valid with restrictions The actual concentration of dissolved test material was not measured analytically.
<b>Flag</b> 16.05.2003	: Critical study for SIDS endpoint (13)
<b>Type</b>	: Other
<b>Species</b>	:
<b>Exposure period</b>	: 96 hour(s)
<b>Unit</b>	: mg/l
<b>LC50</b>	:
<b>Limit test</b>	:
<b>Analytical monitoring</b>	: No
<b>Method</b>	: other
<b>Year</b>	: 2003
<b>GLP</b>	: No
<b>Test substance</b>	: as prescribed by 1.1 - 1.4
<b>Remark</b>	: Inputs to the program are the CAS No. 40601-76-1 and a measured melting point of 160.5 degrees C. The EPIWIN ECOSAR program uses imides and phenols as model analogs for this substance.
<b>Result</b>	: The LC50 values are 3.08 E-8 mg/l based on the imides class and 6.79 E-7 mg/l based on the phenol class.
<b>Reliability</b> 26.05.2003	: (2) valid with restrictions Data were obtained by EPIWIN modeling. (7)

### 4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

<b>Type</b>	: Static
<b>Species</b>	: Daphnia magna (Crustacea)
<b>Exposure period</b>	: 48 hour(s)
<b>Unit</b>	: mg/l
<b>NOEC</b>	: = 1000 measured/nominal
<b>EC50</b>	: > 1000 measured/nominal
<b>Limit Test</b>	: Yes
<b>Analytical monitoring</b>	: No
<b>Method</b>	: OECD Test Guideline 202
<b>Year</b>	: 2002
<b>GLP</b>	: Yes
<b>Test substance</b>	: as prescribed by 1.1 - 1.4
<b>Result</b>	: The rate of mortality in the controls was 5 % (1/20) at 24 hours, and 10% (2/20) at 48 hours. For treated organisms, the mortality rate was 10% at both 24 and 48 hours (2/20). The no effect concentration (NOEC) and LC50 value at 48 hours were 1000 mg whole material/l and > 1000 mg whole material/l, respectively.

**Test condition**

The temperature ranged from 20.1 - 20.9 degrees C during the test. The dissolved oxygen concentrations in the test water ranged from 8.2 - 8.8 mg/l (92-98 % of saturation) at the beginning of the test and 8.0 - 8.4 mg/l (89-93% of saturation) for the remainder of the test. pH values ranged from 7.1 - 7.3 at the beginning of the test and from 7.2 - 7.3 at the end of the test.

: Daphnia magna used in the test were progeny from animals obtained from Aquatic Research Organisms, Hampton New Hampshire in August 2000. A subculture of adults was isolated from these cultures and maintained on site prior to testing. The cultures were fed the green algae, *Selenastrum capricornutum* and a solution prepared from yeast, cereal leaves (*Cerophyll*) and trout chow daily. Less than 24 hours prior to testing, the adults were placed in food-free dilution water. Neonates (< 24 hours old) were collected on July 2, 2002. These organisms were cultured and isolated in moderately hard fresh water (pH 6.5 - 8.0, temperature 20 +/- 2 degrees C), which originated from Jupiter Florida. Water was treated by vigorous aeration, filtered to 20 microns, passed through activated carbon and re-aerated prior to use. The hardness (as calcium carbonate), alkalinity (as calcium carbonate) and specific conductivity were 76 mg/l, 31 mg/l and 407 microSiemens, respectively. Chemical characteristics were listed in an Appendix to the study. No ephippia were produced during culture and the organisms appeared in good physical condition at test initiation.

A 48-hr preliminary range-finding test was conducted at nominal concentrations of 0.0 (control), 0.2, 1, 11, 99 and 1000 mg whole material/l. Five Daphnia were tested for each condition. None of the organisms exposed to concentrations < 1000 mg/l died in the experiment. Twenty percent of Daphnia exposed to 1000 mg/l died. The test material was insoluble at each concentration used.

Due to the insolubility of the test material in the preliminary test, the definitive test was initiated as a limit test with 1000 mg whole material/l. Test water was prepared by directly adding the test material (0.5 g) to 0.5 liters of dilution water. The test vessel was placed onto a mechanical stir plate with a rotating stir bar. The test material was spiked directly into the vortex and allowed to mix for approximately 30 minutes. The liquid was then filtered through a Gelman minicapsule (0.45 microns) Versapore membrane filter. The test filtrate (which appeared clear) was transferred into a glass test chamber, placed onto a 20 degrees C water table, and allowed to reach the desired test temperature (20 +/- 1 degrees C). A control solution containing dilution water only also was prepared.

After initial water quality parameters (temperature, pH, dissolved oxygen) were measured, 5 organisms (one or two at a time) were added to each test chamber. Four replicate chambers were established for the control and test solutions (for a total of 20 control and test organisms). The test chambers were 50-ml glass vials (2.5 cm x 9.5 cm) containing 50 ml of dilution water or test solution (which provided a final water depth of 9.5 cm). All chambers were covered to reduce evaporation. The chambers were placed in a water bath and maintained under a photoperiod of 16 hours light/8 hours darkness (at 9.5-10.6 micromoles/m<sup>2</sup>/sec light intensity). Organisms were not fed during the test and the water was not aerated. Survival and condition of Daphnia and water quality were monitored daily. The test was terminated after 48-hr evaluations had been performed.

The no effect concentration (NOEC) and EC50 values were to be calculated using ToxCalc Comprehensive Toxicity Data Analysis and Database Software (Version 5.0, 1996).

**Test substance**

: The test material was Cyanox 1790, used as supplied by the manufacturer. According to the MSDS, the purity of this material is 93.7-98.9%.

## 4. Ecotoxicity

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<b>Reliability</b>	:	Impurities are not listed on the MSDS. (2) valid with restrictions The actual concentration of dissolved test material was not measured analytically.	
<b>Flag</b> 26.05.2003	:	Critical study for SIDS endpoint	(14)
<b>Type</b>	:	other	
<b>Species</b>	:		
<b>Exposure period</b>	:	48 hour(s)	
<b>Unit</b>	:	mg/l	
<b>EC50</b>	:	.000139 calculated	
<b>Analytical monitoring</b>	:	No	
<b>Method</b>	:	other	
<b>Year</b>	:	2003	
<b>GLP</b>	:	No	
<b>Test substance</b>	:	as prescribed by 1.1 - 1.4	
<b>Remark</b>	:	Inputs to the program are CAS No. 40601-76-1 and a measured melting point of 160.5 degrees C. EPIWIN ECOSAR program chooses phenols as the model analog for this substance.	
<b>Reliability</b> 26.05.2003	:	(2) valid with restrictions Data were obtained by EPIWIN modeling.	(7)

### 4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

<b>Species</b>	:		
<b>Endpoint</b>	:	other	
<b>Exposure period</b>	:	96 hour(s)	
<b>Unit</b>	:	mg/l	
<b>EC50</b>	:	6.14E-10 calculated	
<b>Remark</b>	:	Inputs to the program are CAS No. 40601-76-1 and a measured melting point of 160.5 degrees C. EPIWIN ECOSAR Program chooses phenols as the model analog for this substance.	
<b>Reliability</b>	:	(2) valid with restrictions Data were obtained by EPIWIN modeling.	
<b>Flag</b> 26.05.2003	:	Critical study for SIDS endpoint	(7)

### 4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

#### 4.5.1 CHRONIC TOXICITY TO FISH

#### 4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

#### 4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

#### 4.6.2 TOXICITY TO TERRESTRIAL PLANTS

**4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS**

**4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES**

**4.7 BIOLOGICAL EFFECTS MONITORING**

**4.8 BIOTRANSFORMATION AND KINETICS**

**4.9 ADDITIONAL REMARKS**

## 5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

## 5.1.1 ACUTE ORAL TOXICITY

<b>Type</b>	:	LD50
<b>Value</b>	:	> 10000 mg/kg bw
<b>Species</b>	:	rat
<b>Strain</b>	:	Wistar
<b>Sex</b>	:	male
<b>Number of animals</b>	:	10
<b>Vehicle</b>	:	other: 0.25% agar and 0.10% Tween 80
<b>Doses</b>	:	10000 mg/kg
<b>Method</b>	:	other
<b>Year</b>	:	1973
<b>GLP</b>	:	no
<b>Test substance</b>	:	as prescribed by 1.1 - 1.4
<b>Result</b>	:	None of the animals died and there were no signs of intoxication. Animals gained an average of 106 g over the course of the study. All organs appeared normal at necropsy.
<b>Test condition</b>	:	Ten nonfasted male Harlan-Wistar albino rats (90-120g) were treated with a single oral dose of 10.0 g/kg. The dosing solution contained 0.2 g test material in a total volume of 1 ml of 0.25% agar and 0.10% Tween 80. Animals were observed for toxicity over a period of 14 days, after which they were euthanized and subjected to gross necropsy.
<b>Test substance</b>	:	The test material was Antioxidant A-1790 (Cyanox 1790), used as supplied by the manufacturer. According a data sheet supplied with the study, the purity of the material was 95% and the melting point was 145-148 degrees C. Impurities were not listed.
<b>Reliability</b>	:	(2) valid with restrictions Females were not tested.
<b>Flag</b>	:	Critical study for SIDS endpoint
16.05.2003		(2)

## 5.1.2 ACUTE INHALATION TOXICITY

<b>Type</b>	:	LC50
<b>Value</b>	:	> 20 mg/l
<b>Species</b>	:	rat
<b>Strain</b>	:	
<b>Sex</b>	:	
<b>Number of animals</b>	:	
<b>Vehicle</b>	:	
<b>Doses</b>	:	
<b>Exposure time</b>	:	4 hour(s)
<b>Method</b>	:	other
<b>Year</b>	:	2001
<b>GLP</b>	:	no data
<b>Test substance</b>	:	as prescribed by 1.1 - 1.4
<b>Test condition</b>	:	Value was estimated by an unknown method.
<b>Reliability</b>	:	(4) not assignable No test conditions were noted.
26.05.2003		(3)

## 5. Toxicity

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### 5.1.3 ACUTE DERMAL TOXICITY

**Type** : LD50  
**Value** : > 5000 mg/kg bw  
**Species** : rabbit  
**Strain** : other: albino  
**Sex** : male  
**Number of animals** : 5  
**Vehicle** : water  
**Doses** : 5000 mg/kg  
**Method** : other  
**Year** : 1973  
**GLP** : no  
**Test substance** : as prescribed by 1.1 - 1.4

**Result** : There were no deaths or signs of intoxication or irritation. Animals gained an average of 264 g over the course of the study. There were no remarkable necropsy findings.

**Test condition** : Test material was applied to clipped skin of 5 male albino rabbits (avg. wt 2492 g). The material that was applied was weighed onto Vinylite and moistened with a volume of water equal to two times the weight of the test material). The test site was then covered (the material used was not listed). Animals were observed for 14 days, after which they were weighed, euthanized and subjected to gross necropsy.

**Test substance** : The test material was Antioxidant A-1790 (Cyanox 1790), used as supplied by the manufacturer. According a data sheet supplied with the study, the purity of the material was 95% and the melting point was 145-148 degrees C. Impurities were not listed.

**Reliability** : (2) valid with restrictions  
Females were not tested.

16.05.2003

(2)

### 5.1.4 ACUTE TOXICITY, OTHER ROUTES

#### 5.2.1 SKIN IRRITATION

**Species** : human  
**Concentration** : 2.5 %  
**Exposure** : Semioclusive  
**Exposure time** :  
**Number of animals** : 100  
**Vehicle** : petrolatum  
**PDII** :  
**Result** : not irritating  
**Classification** :  
**Method** : other: modified Draize-Shelanski Repeat Insult Patch Test  
**Year** : 1976  
**GLP** : no data  
**Test substance** : as prescribed by 1.1 - 1.4

**Remark** : It is peculiar that the results state that there were 7 days between induction and challenge, and results of 0 are also listed for days 8, 9, and 10 after induction.

**Result** : All subjects had scores of 0 1-10 days after induction and 24 and 48 hours after challenge. The material was therefore neither irritating nor sensitizing.

**Test condition** : One hundred healthy adults (61 females, 39 males, 18-50 years of age, 58

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black, 2 Puerto Rican, 40 white) served as subjects for the study. A 15 mm patch of test material (2.5% in white petrolatum) was applied to patch sites on the backs or volar forearms of the subjects for 10 alternate-day 24 hour periods under occlusion. Following a seven day test period (in which subjects were evaluated daily), 15 mm challenge patches (1.0% in white petrolatum) were applied in the same manner to fresh sites on the backs or volar forearms of all subjects for 24 hours. Challenge sites were read on removal of the patch and 24 hours thereafter.

**Test substance** : The test material was Antioxidant A-1790 (Cyanox 1790), used as supplied by the manufacturer. According a data sheet supplied with the study, the melting point was 159-161 degrees C. Impurities were not listed.

**Reliability** : (2) valid with restrictions  
The scoring system was not described.

16.05.2003 (16)

### 5.2.2 EYE IRRITATION

**Species** : rabbit  
**Concentration** :  
**Dose** : 100 other: mg  
**Exposure time** :  
**Comment** :  
**Number of animals** : 6  
**Vehicle** :  
**Result** : not irritating  
**Classification** :  
**Method** : other: modified FHSA procedure  
**Year** : 1973  
**GLP** : no  
**Test substance** : as prescribed by 1.1 - 1.4

**Remark** : It is unclear how the eyes were scored. No scores were provided.  
**Result** : There was no irritation of the cornea, iris or conjunctiva at 24, 48 or 72 hours in any of the eyes.

**Test condition** : Test material (100 mg) was applied to one eye of each of 6 rabbits. Eyes were evaluated 24, 48 and 72 hours after treatment.

**Test substance** : The test material was Antioxidant A-1790 (Cyanox 1790), used as supplied by the manufacturer. According a data sheet supplied with the study, the purity of the material was 95% and the melting point was 145-148 degrees C. Impurities were not listed.

**Reliability** : (2) valid with restrictions  
The study is not well documented.

16.05.2003 (2)

### 5.3 SENSITIZATION

**Type** : other: modified Draize-Shelanski Repeat Insult Patch Test  
**Species** : human  
**Concentration** : 1<sup>st</sup>: Induction 2.5 %  
2<sup>nd</sup>: Challenge 1 %  
3<sup>rd</sup>:  
**Number of animals** : 100  
**Vehicle** :  
**Result** : not sensitizing  
**Classification** :  
**Method** : other  
**Year** : 1976  
**GLP** : no data

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**Test substance** : as prescribed by 1.1 - 1.4

**Remark** : It is peculiar that the results state that there were 7 days between induction and challenge, and results of 0 are also listed for days 8, 9, and 10 after induction.

**Result** : All subjects had scores of 0, 1-10 days after induction and 24 and 48 hours after challenge. The material was therefore neither irritating nor sensitizing.

**Test condition** : One hundred healthy adults (61 females, 39 males, 18-50 years of age, 58 black, 2 Puerto Rican, 40 white) served as subjects for the study. A 15 mm patch of test material (2.5% in white petrolatum) was applied to patch sites on the backs or volar forearms of the subjects for 10 alternate-day 24 hour periods under occlusion. Following a seven day test period (in which subjects were evaluated daily), 15 mm challenge patches (1.0% in white petrolatum) were applied in the same manner to fresh sites on the backs or volar forearms of all subjects for 24 hours. Challenge sites were read on removal of the patch and 24 hours thereafter.

**Test substance** : The test material was Antioxidant A-1790 (Cyanox 1790), used as supplied by the manufacturer. According a data sheet supplied with the study, the melting point was 159-161 degrees C. Impurities were not listed.

**Reliability** : (2) valid with restrictions  
The scoring system was not described.

16.05.2003 (16)

### 5.4 REPEATED DOSE TOXICITY

**Type** : Sub-chronic

**Species** : rat

**Sex** : male/female

**Strain** : Sprague-Dawley

**Route of admin.** : oral feed

**Exposure period** : 90 days

**Frequency of treatm.** : continuous

**Post exposure period** : not applicable

**Doses** : 25, 100 and 400 mg/kg

**Control group** : yes, concurrent no treatment

**NOAEL** : = 400 mg/kg bw

**Method** : other

**Year** : 1977

**GLP** : no

**Test substance** : as prescribed by 1.1 - 1.4

**Result** : One animal in the mid-dose group (100 mg/kg) died after 12 days of treatment. Study personnel did not attribute this death to administration of test material. Animals were generally healthy during the study, but exhibited alopecia around the nares and paws, diarrhea, watery eyes (animals that were bled, only) and encrustment around the nares. Study personnel attributed these signs to ingestion of a powdered food and did not consider them to be related to test material. Other than week one (where females treated with 25 mg/kg test material ate less food than females in other groups) and week 3 (when males in the 25 mg/kg group ate less food than males in the other groups), there was no difference between food intake of treated and control animals. There was no effect of treatment on weight or weight gain.

Gamma-glutamyl transpeptidase (GGTP) values in males treated with 400 mg/kg were higher than control at 90 days (4.1 vs. 2.6 IU/l), but were within historical limits. The elevation was due to 2/5 males that had GGTP values that were twice the values of the other males. Glucose values in females treated with 100 and 400 mg/kg (163 and 162 mg/dl, respectively) were

lower than controls (209 mg/dl) at 90 days but were within the range of historical values (90-284 mg/dl). The significant differences in glucose between high and mid dose females and controls were considered to be a result of some unusually high values in the controls and were not considered to be related to test material.

At 90 days, there was a trend toward increased red blood cells in low ( $8.27 \times 10^6/\text{mm}^3$ ) and mid-dose animals [ $(8.63 \times 10^6/\text{mm}^3$ , value significantly different from study control ( $7.99 \times 10^6/\text{mm}^3$ ) and historical controls]; however, counts in high dose animals ( $8.04 \times 10^6/\text{mm}^3$ ) were similar to controls. Since the increase was not dose-related, this observation was thought to be artifactual in nature and not related to administration of test material.

There was no effect of treatment on urinalysis, organ weights, or gross pathology. One of the males with a high GGTP value had focal perivascular and periductal mononuclear leukocyte infiltration in the liver, but the other animal did not demonstrate any liver pathology. Livers of other males in the high dose group had histopathology similar to that of controls. Other mild inflammatory lesions characterized by leukocytic infiltration also occurred in the lungs, liver and kidneys of a few animals in each group. All changes were considered to be spontaneous and not related to administration of test material.

**Test condition**

: COBS Sprague-Dawley rats (80 per sex) were acclimated for 8 days before treatment. They were then assigned to 4 test groups [0 mg/kg bw (control diet), or 25, 100 or 400 mg/kg bw test material] of 20 per sex per group by weight stratification. At time of assignment, all animals were 4 weeks old. Weights of male and female animals ranged from 69-100 g and 64-95 g, respectively. Animals were individually housed in suspended stainless steel cages and were maintained in a room that was kept at 70-75 degrees F and 40-50% relative humidity, with 10 exchanges of air/hour. The light cycle was 12 hr light/12 hours dark.

Test diets were prepared by mixing the appropriate amount of test material with equal amounts of ground Purina Lab Chow in a high speed blender (blending time was not noted). After blending, an additional 2 parts of ground lab chow was mixed by hand into the blended material. After this step, an additional amount of ground lab chow was added to give the appropriate dose level (25 mg/kg bw, 100 mg/kg bw or 400 mg/kg bw). The diet was then placed in a twin-shell mixer and mixed until the test material was distributed throughout the diet. The formula used to determine the amount of material added to feed was as follows: mean body weight x dietary level x 7 days/mean weekly food consumption = mg test material/kg feed. Fresh diets were prepared at least once per week. Doses were adjusted weekly on the basis of the weight and food consumption for the preceding week. Doses were not adjusted for purity of the material.

Animals were maintained on their respective diets for a period of 90 days. As mentioned above, food consumption and body weights were measured weekly. Animals selected for clinical chemistry, hematology and urinalysis (5 per sex per group) were determined by protocol and a table of randomization. Clinical chemistry (gamma-glutamyl transpeptidase, glucose, glutamic-oxaloacetic transaminase, glutamic pyruvic transaminase, and blood urea nitrogen), hematology (erythrocyte count, hematocrit, hemoglobin, differential and total leukocyte count, and platelet count) and urinalyses (appearance, glucose, microscopic examination of sediment, pH, protein and specific gravity) were performed on the same animals at appropriate time intervals (day 90 for clinical chemistries, days 45 and 90 for hematologies). Animals were fasted overnight before blood was collected from the orbital sinus on day 45 and from an unknown site on day 90. Urine also was collected on days 45 and 90. Organ weights (brain, gonads, heart, kidneys and liver) were measured in all animals that

survived to day 90.

At study termination, all animals were weighed. They were then euthanized. The urogenital orifices, tail, each pinna, eyes and external auditory meatus were examined visually and by palpation for distortions. All subcutaneous tissues were examined including regional lymph nodes, mammary and salivary glands. Abdominal contents, and the brain, pituitary gland and cranial nerves also were examined grossly. The following tissues were taken and preserved for possible histologic evaluation: adrenal, aorta, urinary bladder, bone, bone marrow, cerebellum, cerebrum, pancreas, pituitary, skin, stomach, thyroid, colon, esophagus, eye, heart, ileum, jejunum, kidney, prostate, salivary gland, spleen, testes, tongue, uterus, liver, lung, lymph node, mammary gland, muscle (skeletal), sciatic nerve, ovary, parathyroid, seminal vesicle, spinal cord, thymus and trachea. Any organ or tissue exhibiting a lesion was noted and the lesion was taken for histopathological examination. Complete histopathology was performed on 10 animals/sex/group from the control and high dose groups. These animals were selected randomly from a table. Microscopic examination of the heart, liver, lungs and kidneys was performed on all additional animals that survived to termination.

Data for food intake, weight gain, clinical chemistries, hematologies, urinalyses, and organ weights were analyzed using a blocked 2-way analysis of variance that compared data between and within groups. If F values were significant, data were compared using Dunnett's t-test. The level of significance is  $p < 0.05$ .

<b>Test substance Conclusion</b>	: The purity of the test material was 90.0%. Impurities were not listed. : The changes that were observed (elevated gamma-glutamyl transpeptidase in high dose males, decreased glucose in females, and increased red blood cell counts in mid-dose males at 90 days) were not considered to be related to test material since they were not dose-dependent and were within normal limits. Therefore, authors concluded that the "no effect" dose was 400 mg/kg bw.
<b>Reliability</b>	: (2) valid with restrictions Doses of test material, homogeneous distribution, and stability in the diet were not confirmed analytically. More hematological, clinical chemistry and urinalysis parameters are measured in guideline studies. A high enough dose to produce a significant toxicological effect was not used.
<b>Flag</b> 22.05.2003	: Critical study for SIDS endpoint <span style="float: right;">(20)</span>
<b>Type</b>	: Sub-chronic
<b>Species</b>	: dog
<b>Sex</b>	: male/female
<b>Strain</b>	: Beagle
<b>Route of admin.</b>	: oral feed
<b>Exposure period</b>	: 13 weeks (91 days)
<b>Frequency of treatm.</b>	: 1 hour/day
<b>Post exposure period</b>	: not applicable
<b>Doses</b>	: 46 mg/kg/day
<b>Control group</b>	: yes, concurrent no treatment
<b>NOAEL</b>	: = 46 mg/kg bw
<b>Method</b>	: other
<b>Year</b>	: 1983
<b>GLP</b>	: yes
<b>Test substance</b>	: as prescribed by 1.1 - 1.4

**Result** : None of the controls or animals treated with CL 226,034 died. Clinical signs such as unilateral or bilateral serous ocular discharge, undigested food (occasionally mixed with mucoid substance) found in the tray after feeding, loose feces (occasionally mixed with mucoid substance) found in tray before and after feeding, redness of the pinnae, and areas of skin

redness and partial alopecia were noted in both control and treated animals at similar incidences.

Throughout the treatment period, the mean body weights of CL 226,034-treated dogs of both sexes were consistently lower than the controls, but none of the values were statistically significant. The mean body weight gains of control males and females were 2.4 and 2.3 kg, respectively, and treated males and females gained an average of 2.0 and 1.7 kg, respectively. Throughout the experiment, the average food intake of treated animals was slightly but consistently lower than that of treated dogs. None of the values were significantly different in males. Average food intake of treated females was significantly different from controls at week -3 (217.2 g/dog/day in control vs. 160.4 g/dog/day in treated), week 1 (310.7 g/dog/day in control vs. 275.5 g/dog/day in treated), and week 2 (330.6 g/dog/day in control vs. 288.3 g/dog/day in treated).

The test material was stable in the diet for 14 days and was distributed homogeneously. The amount of test material intake in males and females ranged from 88.4-103.7% and 99.4 - 110.0% of target, respectively. The mean amount of CL 226,034 intake for the entire feeding period was 97.5 +/- 4.41% of the desired amount for males and 103.8 +/-3.64% for females.

The ophthalmologic examinations of 2 female dogs (one control and one treated) were abnormal during the test. One control had a right eye with a small focus of tapetal hyperelectivity and pigment clumping lateral to the main retinal vessels at pretreatment, which was not evident at week 6. At week 12, the right eye had a small area of tapetal hyperelectivity that appeared to be a focal inactive scar. In a treated animal that had normal exams at pretreatment and week 6, the right eye had a small hemorrhage in the nerve fiber layer, superolateral to the optic nerve head at week 12. The significance of these findings was not listed.

Throughout the study, there was no consistent effect of test material on any hematological, clinical biochemical or urinalysis parameter. Occasional differences in parameters were judged to be inconsequential since the values were within normally accepted ranges, did not change consistently over time and were not observed in both sexes.

There was no effect of treatment on weight of any organ examined. Gross pathological findings revealed that 4 dogs (1 male and female in each of the groups) had roundworms in the intestines. Other gross findings were judged to be incidental. Two controls showed excoriation and/or reddening of the skin. Upon histological examination, one of the animals had demodectic mange, and the other showed slight inflammatory changes. Another control dog that did not exhibit gross skin changes also had demodectic mange upon histologic examination.

Histopathological examinations did not reveal any treatment-related effects. One treated male had a small granuloma and a focus of eosinophils in the mesenteric lymph nodes. It was concluded that this was probably associated with parasitic larval migration. Parafollicular cell hyperplasia in the thyroid was found in 3 treated animals and 3 controls. One control and one treated male had macrophages containing a dark brown pigment in the paracortical zone of 1 mandibular lymph node. A focus of capsular thickening seen in the spleen of a treated female was judged to be incidental.

**Test condition**

- : Purebred beagle dogs (31/sex, approximately 5 months old) were acclimated for 55 days before use. The supplier had immunized the dogs against canine distemper, infectious canine hepatitis, canine leptospirosis, parovirus and rabies. A veterinarian concluded that they were healthy prior to shipment. The body weight range of the animals just prior to treatment was 5.6 to 9.3 kg for males and 4.5 to 8.5 kg for females. The dogs were

housed individually in a room maintained under a 12 hr light/12 hr dark cycle and 12 air changes/hour.

During the acclimation period, the animals were randomly assigned to 7 treatment groups of 4 animals/sex. Five of these groups were going to be treated with a compound B (which was not identified). One group served as a control group, and the other was to be treated with CL226,034 (Cyanox 1790).

Diets containing the test material were prepared weekly by adding the required weight of the test material to known amounts of powdered certified dog chow and mixing for approximately 30 minutes in a Hobart mixer. Homogeneity and adequacy of mixing of the test diets were evaluated by analyzing samples from the top, middle and bottom of the mixer. Stability of the test diet was analyzed immediately after preparation and storage at ambient temperature for 7 and 14 days. Fresh diets were stored at approximately 4 degrees C in labeled airtight plastic containers for the period of use. The concentration of test material in the diets was checked during weeks 1, 2, 4, 8 and 13, immediately after preparation.

From study day -5.1 to 0.1, the dogs were fed 400 g/day of a standard certified commercial dog food. From study day 0.2 to termination, the males were fed 450 g/day and the females 400 g/day. For one hour per day, test animals were fed diet containing test material (compound B or 46 mg/kg/day CL226,034). Fresh water was available ad libitum. There were no contaminants in the food or water that were expected to affect the outcome of the test. All controls and animals treated with CL226,034 were on test for a minimum of 91 days.

Animals were observed for clinical signs twice daily (or more frequently if necessary). Animals that exhibited overt changes in physical appearance or behavior were examined by a veterinary aide. Abnormal animals were examined by a veterinarian. Each animal was weighed weekly prior to and during the treatment period, and just prior to necropsy. Food consumption was determined daily and calculated and documented weekly. An ophthalmoscopic examination was conducted prior to treatment and during weeks 6 and 12 of treatment on all controls and animals treated with CL226,034. Atropine sulfate (1% solution) was administered as a mydriatic, and the conjunctivae, sclera, cornea, iris and fundus were examined by a veterinary ophthalmologist.

Laboratory investigations (hematologies, clinical biochemistries and urinalysis) were performed on all controls and animals treated with CL226,034 prior to treatment and during weeks 6 and 12. Blood samples were collected from the jugular veins after animals were fasted overnight. Urine samples were collected over the final 17 hours of a 21-hour period food and water deprivation. The hematological parameters measured were hemoglobin, hematocrit, red blood cell count, white blood cell count (total and differential), platelet count, prothrombin time, and Wintrobe's constants). Clinical biochemistries were blood urea nitrogen, glucose, total protein, albumin (A), globulin (G), A/G ratio, serum sodium, potassium, calcium and chloride, creatinine, serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), serum alkaline phosphatase (SAP), lactate dehydrogenase (LDH) and total bilirubin. Urinalyses included volume, specific gravity, pH, protein, color and appearance, hemoglobin, nitrite, glucose, urobilinogen, ketones, microscopy of centrifuged deposit and bile pigments.

A complete gross necropsy was performed on each dog that survived to study termination. The animals were euthanized and exsanguinated, and the following organs were dissected and weighed: adrenals, brain, heart,

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kidneys, liver, ovaries, pituitary, spleen, testes and thyroid lobes (with parathyroids). Paired organs were weighed separately. The following organs were preserved, sectioned, stained and subjected to histopathological examination: adrenals, aorta (thoracic), bone (sternum with marrow, brain (cerebellum, cerebrum and brain stem), cecum, colon, duodenum, epididymides, esophagus, eyes, jejunum, gallbladder, heart, ileum, kidneys, liver (sample from 2 lobes), lungs, lymph nodes (mandibular and mesenteric), mammary gland (inguinal), optic nerves, ovaries, pancreas, pituitary, prostate, salivary gland (mandibular), sciatic nerve, skeletal muscle, skin, spinal cord (cervical), spleen, stomach (cardiac region, fundus and pyloric region), testes, thymus, thyroid lobes (and parathyroids), tongue, trachea, urinary bladder, uterus (horns and body), vagina and any abnormalities. Femoral bone marrow smears were prepared for each animal and were stained with May-Grunwald-Giemsa. These smears were to be evaluated if hematological findings were abnormal.

Mean values and standard deviations of data (with the exception of hematological and blood biochemical data) were statistically analyzed using Student's t-test. The probability of t was calculated at the  $p < 0.5$  value, and any significant differences were noted.

Hematological and blood biochemical data were analyzed using Bartlett's test followed by Student's t-test when the variance between groups was homogeneous or by the Kruskal-Wallis test when the variance between groups was heterogeneous.

**Test substance** : The test material was CL 226, 034, which is Cyanox 1790. It was supplied as a white powder from American Cyanamid. Documentation of purity did not accompany the study. According to a Cytec Industries Inc. (formerly known as American Cyanamid) MSDS of the material written in 2001, the purity of the material is 93.7 - 98.9%. Impurities are not listed on the MSDS.

**Reliability** : (2) valid with restrictions  
Only one dose of material was tested. An additional dose that was expected to produce toxicity should have been tested. Otherwise, the study was comparable to a guideline study.

**Flag** : Critical study for SIDS endpoint

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(21)

**Type** : Sub-chronic  
**Species** : dog  
**Sex** : male/female  
**Strain** : Beagle  
**Route of admin.** : oral feed  
**Exposure period** : 90 days  
**Frequency of treatm.** : continuous  
**Post exposure period** : not applicable  
**Doses** : 7.5, 15 and 30 mg/kg  
**Control group** : yes, concurrent no treatment  
**NOAEL** : = 30 mg/kg bw  
**Method** : other  
**Year** : 1977  
**GLP** : no  
**Test substance** : as prescribed by 1.1 - 1.4

**Remark** : The no effect level assigned by study personnel was 30 mg/kg. They did not consider the reduced body weight gains in females to be related to test material because 1) food consumption was comparable between groups and 2) no abnormal clinical signs were noted.

**Result** : None of the animals died during the study. During the treatment period, variations in stool consistency (soft stool, diarrhea, etc.) were noted intermittently in treated animals. This observation was attributed to the

introduction of a foreign material into the GI tract of the beagle and was not considered to be related to the administration of Cyanox 1790 specifically. There were no significant differences in food consumption between controls and animals treated with test material. All animals gained or maintained body weight. There were no significant differences between weights of male control and treated dogs except at week 0 (before treatment). Female dogs treated with any dose of test material had lower body weights than controls throughout the study (including week 0). Weight gains of females decreased in a dose dependent manner, with the average gains for females treated with 0, 7.5, 15, or 30 mg/kg being 1.6, 1.3, 1.1, and 0.7 (units were not listed, but are assumed to be kg).

At the 30 day interval, dogs treated with 7.5, 15 or 30 mg/kg test material (112, 112 and 119 mg/dl in males and 114, 124 and 119 mg/dl in females, respectively) had significantly lower glucose values than dogs in the control group (140 and 133 mg/dl in males and females, respectively). At 90 days, female dogs treated with 7.5 and 30 mg/kg had lower glutamic-oxaloacetic transaminase values (27 and 26 IU/l) than controls (32 IU/l). At 90 days, the erythrocyte count was significantly lower in male dogs treated with 15 mg/kg than controls (5.47 vs. 6.11 x 10E6/mm<sup>3</sup>, respectively). As all values were within normal limits, these observations were not considered to be related to treatment. No other differences in any clinical chemistry or hematological parameter and no changes in any urinalysis parameter were noted between groups.

There was no effect of treatment on weight of any organ examined or on gross or histopathology. All changes observed were considered to be related to necropsy procedures or a parasitic infection.

**Test condition**

: Thirty two pure bred Beagle dogs (16 per sex) were acclimated for 16 days before treatment. They were vaccinated against canine distemper, hepatitis and leptospirosis by the supplier. Animals were individually housed in suspended stainless steel cages and were maintained in a room that was kept at 70-76 degrees F and 25 - 50% relative humidity. The light cycle was 12 hr light/12 hours dark. Cages were cleaned semi-monthly.

Dogs were assigned to 4 test groups [0 mg/kg bw (control diet), or 7.5, 15 or 30 mg/kg bw test material] of 4 per sex per group one week prior to treatment by weight stratification. On day 1 of treatment, weights and ages of the animals were 5.1 - 6.7 kg and 4 - 5 months, respectively.

Test diets were prepared by geometrically diluting the appropriate amount of test material with Purina Laboratory Canine Diet in a grounded twin shell blender. The mixing time was not mentioned. Diets containing the least amount of test material were mixed first. The blender was completely cleaned between mixing each dose level. The formula used to determine the amount of material added to feed was as follows: mean body weight (g) x dose (mg/kg bw)/mean food consumption per day (g) = mg test material/kg diet. The desired doses were 7.5, 15.0 and 30.0 mg/kg bw. Doses were not adjusted for purity of the material. Doses were adjusted weekly based on the group body weights of dogs for that week. Diets were prepared weekly.

Animals were maintained on their respective diets for a period of 90 days. As mentioned above, food consumption and body weights were measured weekly. Clinical chemistry (gamma-glutamyl transpeptidase, glucose, glutamic-oxaloacetic transaminase, glutamic pyruvic transaminase, and blood urea nitrogen), hematology (erythrocyte count, hematocrit, hemoglobin, differential and total leukocyte count, and platelet count) and urinalyses (appearance, glucose, microscopic examination of sediment, pH, protein and specific gravity) were performed on blood and urine collected from all animals after a 16 hour fast during the week prior to treatment, and weeks 8 and 13 of treatment. The site of blood collection

was not mentioned.

At study termination (days 90 and 9a), all animals were weighed. They were then euthanized and exsanguinated. The urogenital orifices, anus, tail, external auditory meatus and skin were examined visually and by palpation for masses. All subcutaneous tissues were examined including regional lymph nodes, mammary and salivary glands. The brain, pituitary gland, spinal cord, eyes and cranial nerves also were examined grossly. The entire gastro-intestinal tract from the cephalad part of the esophagus to the caudal part of the colon was incised, washed with tap water, and the mucosa was subjected to visual examination. The condition of the thoracic viscera was noted including the thymus and lymph nodes. The lungs were removed and all lobes examined, and the heart was excised to permit examination of each chamber. The brain, gonads, heart, kidneys, pituitary, thyroids and liver of all animals were weighed. The liver and kidneys were sectioned at varying intervals.

Small portions of the following tissues were taken and preserved in 10% neutrally buffered formalin for possible histologic evaluation: adrenal, aorta, gall bladder, urinary bladder, bone, bone marrow, cerebellum, cerebrum, colon, esophagus, eye, heart, ileum, jejunum, kidney, liver, lung, lymph node, mammary gland, muscle (skeletal), sciatic nerve, ovary, pancreas, parathyroid, pituitary, prostate, salivary gland, skin, spinal cord, spleen, stomach, testis, thymus, thyroid, tongue, trachea and uterus. Sections were prepared at 5 microns from paraffin blocks and stained with hematoxylin and eosin.

Data for food intake, body weight, clinical chemistries, hematologies, and organ weights were analyzed using a randomized block analysis of variance. The level of significance is  $p < 0.05$ . No statistical evaluations were performed on survival or urinalysis data and incidences of gross or microscopic lesions.

<b>Test substance</b>	:	The page describing the purity of the test material was missing from the report. However, the purity of the test material used in a rat study that was performed at the same facility around the same time was 90.0%. Impurities were not listed.
<b>Reliability</b>	:	(2) valid with restrictions Doses of test material, homogeneous distribution, and stability in the diet were not confirmed analytically. More hematological, clinical chemistry and urinalysis parameters are measured in guideline studies. A dose that produced a significant toxicological effect also should have been tested.
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<b>Type</b>	:	Sub-chronic
<b>Species</b>	:	rat
<b>Sex</b>	:	male/female
<b>Strain</b>	:	Sprague-Dawley
<b>Route of admin.</b>	:	oral feed
<b>Exposure period</b>	:	30 days
<b>Frequency of treatm.</b>	:	continuous
<b>Post exposure period</b>	:	not applicable
<b>Doses</b>	:	0.5, 1.0 and 2.0 %
<b>Control group</b>	:	yes, concurrent no treatment
<b>NOAEL</b>	:	= 2 %
<b>Method</b>	:	other
<b>Year</b>	:	1976
<b>GLP</b>	:	no
<b>Test substance</b>	:	as prescribed by 1.1 - 1.4
<b>Remark</b>	:	This was a range-finding study for a 90-day study. Therefore, it was not designed to be an in-depth examination. It is likely that the increased relative liver weight in the high dose females is due to reduced body

weight. The authors did not consider the decreased body weight of high dose females or the changes observed in the liver of one high dose male to be indicative of a treatment-related effect. They therefore determined that the no effect level was 2%. However, it is interesting to note that high dose females had lower body weights but increased food consumption. Therefore, it appears that the material did have an effect on body weight at the highest dose. It was noted in the text that diarrhea occurred in high dose (as well as low dose animals). No explanation was given for this observation, and it was not considered to be significant by study personnel.

**Result**

Based on the results, doses of 100, 400 and 1600 mg/kg were assigned for the 90-day study. However, the highest dose actually used in the 90-day study was 400 mg/kg.

: None of the animals died. Rats receiving test material exhibited diarrhea (low and high-dose animals) and irritation of the face, ears and stomach (mid and high dose animals). Because similar irritation is occasionally observed in Sprague-Dawley rats it was not attributed to administration of test material. Alopecia was noted in controls as well as treated animals. A significant effect of treatment on food consumption at week 4 was noted in females. There was a trend towards increased food consumption in all treated groups, with the value for the high-dose females significantly different from control. The average values for food consumption of females treated with 0, 0.5, 1.0 and 2.0% test material were 118, 133, 131 and 138 g/week respectively. The authors concluded that the increase in food consumption was not related to administration of test material.

Although the authors stated there was no effect on body weight, there was a trend towards decreased terminal body weight in females at the two highest doses, with the average value for the high dose group significantly different from control. The average values for terminal body weight of females treated with 0, 0.5, 1.0 and 2.0% test material were 199, 208, 173 and 159 g/week respectively.

There was no effect of treatment on absolute organ weights. However, in females, average relative liver weights of animals treated with 0.5% (4.98), 1.0% (5.97) and 2.0% (6.98) and average relative kidney weights of animals treated with 2.0% test material (1.207) were significantly different from control values for liver (4.67) and kidney (0.831).

**Test condition**

The only significant gross observation seen at necropsy was discoloration of the liver of 2 high dose males and 2 high dose females. No histopathological changes were observed in 3/4 of these rats. One high dose male exhibited minimal intralobular scattered foci of extra medullary hematopoiesis and mononuclear infiltrates. Infrequent degenerating individual hepatocytes were associated with the mononuclear infiltrates. It was concluded that the effects on the liver were not due to test material.

: Forty healthy CD rats ( $\leq$  4 weeks past weaning and  $\leq$  100 g) were acclimated for 7 days prior to treatment. The rats were housed individually and provided food and water ad libitum. Rats were randomly allocated to 4 groups of 5/sex. The groups were given diet containing 0 % (control), 0.5, 1.0 or 2.0 % test material for 30 consecutive days. Details about how the diets were prepared were not present.

All animals were observed daily to detect possible signs of toxicity. Food intake and body weight were measured weekly. Necropsies were performed on all animals at study termination. At necropsy, all animals received a thorough examination including all body surfaces, both internal and external, subcutaneous tissues, and the following organs: adrenal, aorta, urinary bladder, bone, bone marrow, cerebellum, cerebrum, colon, esophagus, eye, heart, ileum, jejunum, kidney, liver, lung, lymph node, mammary gland, skeletal muscle, sciatic nerve, ovary, pancreas, parathyroid, pituitary, prostate, salivary gland, seminal vesicle, skin, spinal

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cord, spleen, stomach, testes, thymus, thyroid, tongue, trachea and uterus. The liver and kidneys (combined) were weighed. The animal carcass also was examined for lesions and other irregularities. The aforementioned organs were preserved in buffered 10% formalin and the livers of 2 high dose male and female rats were examined histologically.

**Test substance** : Data were analyzed using a parametric analysis of variance (randomized block design) and Student's t tests. The level of significance was  $p < 0.05$ . The test material was Cyanox 1790. Documentation of purity did not accompany the study. According to a Cytec Industries Inc. (formerly known as American Cyanamid) MSDS of the material written in 2001, the purity of the material is 93.7 - 98.9%. Impurities are not listed on the MSDS.

**Reliability** : (2) valid with restrictions  
Doses of test material, homogeneous distribution, and stability in the diet were not confirmed analytically. Clinical chemistry, urinalysis and hematological parameters were not measured.

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(18)

**Type** : Sub-chronic  
**Species** : dog  
**Sex** : male/female  
**Strain** : Beagle  
**Route of admin.** : oral feed  
**Exposure period** : 30 days  
**Frequency of treatm.** : continuous  
**Post exposure period** : not applicable  
**Doses** : 25, 100, 400 and 1600 mg/kg  
**Control group** : no  
**NOAEL** : none assigned  
**LOAEL** : = 400 mg/kg bw  
**Method** : other  
**Year** : 1976  
**GLP** : no  
**Test substance** : as prescribed by 1.1 - 1.4

**Remark** : On days 22-30, dogs in the 25, 100 and 400 mg/kg/d groups were inadvertently given 2.1 g test material/kg feed (84.0 mg/kg bw), 8.17 g test material/kg feed (333.3 mg/kg bw), and 26.7 g test material/kg feed (1335 mg/kg bw). The LOAEL listed above was assigned by study personnel. However, since all doses appeared to cause histological changes in the liver, the LOAEL appears to be lower than the lowest doses tested (25-84 mg/kg).

**Result** : None of the animals died. No abnormalities were noted in the general appearance or behavior. Soft feces were noted on various days from days 11-22 in 1/2 animals dosed with all concentrations of test material except the high dose, and 2/2 animals treated with the high dose. The number of days within this interval that the dogs exhibited soft feces increased with increasing concentration.

Both dogs treated with the highest two concentrations of test material (400-1135 and 1600 mg/kg) and one dog in the 100-333.3 mg/kg group lost weight during the study. The other dogs gained weight at an acceptable rate. Weight loss was attributed to be a significant effect of treatment. Food consumption in the highest two dose groups was reduced.

One high dose dog had increased liver, kidney and adrenal weights compared to the other animals and historical controls. Another high dose dog had a slightly increased adrenal weight. One dog in the 100-333.3 mg/kg group had increased kidney weight.

A consistent discoloration and purple stippling of the liver was noted

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(particularly in the high dose animals). The gall bladder of one dog in the 100-333.3 mg/kg group and another in the 1600 mg/kg group appeared thickened and contained bright yellow bile. The dog in the 1600 mg/kg group that had changes in the gallbladder also had an enlarged spleen and dark colored peribronchial nodes. The kidneys of dogs in all groups except the 25-84 mg/kg group had pale cortices and dark pink outer medullae. The cardiac muscle of one high dose dog had a greyish tinged appearance.

The liver, kidney, gallbladder and heart of the animals that exhibited gross changes were examined histologically. The heart, gallbladder and kidneys were normal (with the exception of moderate vacuolization of the proximal tubule epithelium in one 400-1135 ppm female). At 100-333.3 mg/kg and above, there was marked central lobular hepatocyte degeneration. The change was characterized by central lobular fatty degeneration, hydropic swelling of hepatocytes, hepatocyte necrosis and infiltrates of mononuclear leukocytes. Occasional foci of hemorrhage were present. At the 1600 mg/kg concentration, proliferation of fibroblasts was observed. At 25-84 mg/kg, mild hydropic swelling and necrosis and moderate fatty changes were noted in the liver.

**Test condition** : Eight purebred beagle dogs (4/sex) actively immunized against distemper, infectious hepatitis, leptospirosis and rabies were acclimated for 7 days prior to treatment. They rats were housed individually and provided water ad libitum. A daily portion of Purina Laboratory Canine Diet was offered each day. Dogs were randomly allocated to 4 groups of 2/sex. The groups were given diet containing 25, 100, 400 or 1600 mg/kg/day test material for 30 consecutive days.

Test material was mixed with the diet using a Patterson-Kelley Twin Shell Blender at the appropriate concentrations based on the mean body weight of the dogs. A daily portion of 250 g/dog was offered each afternoon from days 0-21 and 300 g/dog from day 22-30.

All animals were observed daily to detect possible signs of toxicity. Special attention was placed on appearance, behavior and excretory functions. Food intake was recorded daily and body weight was measured prior to treatment, weekly during treatment and at termination. Necropsies were performed on all animals at study termination. At termination, all animals were euthanized and exsanguinated. Weights of the brain, gonads, heart, kidneys, liver, pituitary and thyroids were recorded and relative organ weights/body weight were calculated. The adrenal, aorta, gall bladder, urinary bladder, bone, bone marrow, cerebellum, cerebrum, colon, esophagus, eye, heart, ileum, jejunum, kidney, liver, lung, lymph node, mammary gland, skeletal muscle, sciatic nerve, ovary, pancreas, parathyroid, pituitary, prostate, salivary gland, skin, spinal cord, spleen, stomach, testes, thymus, thyroid, tongue, trachea and uterus were preserved in buffered 10% formalin for possible future examination.

**Test substance** : The test material was Cyanox 1790. Documentation of purity did not accompany the study. According to a Cytec Industries Inc. (formerly known as American Cyanamid) MSDS of the material written in 2001, the purity of the material is 93.7 - 98.9%. Impurities are not listed on the MSDS.

**Conclusion** : At 1600 mg/kg, reduced food consumption could have been due to the unappetizing nature of the diet. Necropsy findings and organ weight data indicated possible treatment-related effects on the liver at 400 and 1600 mg/kg.

**Reliability** : (4) not assignable  
Doses of test material, homogeneous distribution, and stability in the diet were not confirmed analytically. Clinical chemistry, urinalysis and hematological parameters were not measured. Only 2 animals were tested per dose. There were no negative controls. The amount of material given to dogs in the 25, 100 and 400 mg/kg/day groups was much higher than

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the desired doses from days 22-30.

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**5.5 GENETIC TOXICITY 'IN VITRO'**

- Type** : Bacterial reverse mutation assay
- System of testing** : S. typhimurium strains TA98, TA100, TA1535, TA1537 and E. coli WP2uvrA
- Test concentration** : 0, 62, 185, 556, 1667 and 5000 micrograms/plate (Test 1); 0, 125, 250, 500, 1000 and 2000 micrograms/plate (Test 2)
- Cytotoxic concentr.** : > 5000 micrograms/plate
- Metabolic activation** : with and without
- Result** : negative
- Method** : OECD Test Guideline 471
- Year** : 2001
- GLP** : yes
- Test substance** : as prescribed by 1.1 - 1.4
- Result** : The test material was not positive in any of the strains tested in the absence and presence of S-9 mix. In test 1, the mean numbers of revertants in negative control cultures of S. typhimurium strains TA98, TA100, TA1535 and TA1537 and E. coli strain WP2uvrA were 31, 139, 14, 11 and 34 without S-9 and 35, 136, 16, 9 and 40 with S-9 (respectively). With the test material, the numbers of revertants in negative control cultures of S. typhimurium strains TA98, TA100, TA1535 and TA1537 and E. coli strain WP2uvrA ranged from 23-32, 103-135, 18-25, 5-9 and 26-37 without S-9 and 34-53, 111-150, 10-15, 6-11 and 21-34 with S-9 (respectively). In test 2, the mean numbers of revertants in negative control cultures of S. typhimurium strains TA98, TA100, TA1535 and TA1537 and E. coli strain WP2uvrA were 36, 164, 19, 19 and 32 without S-9 and 64, 156, 18, 24 and 40 with S-9 (respectively). With the test material, the numbers of revertants in negative control cultures of S. typhimurium strains TA98, TA100, TA1535 and TA1537 and E. coli strain WP2uvrA ranged from 38-45, 160-175, 17-29, 15-22 and 29-36 without S-9 and 54-66, 163-172, 14-25, 20-24 and 36-49 with S-9 (respectively).
- The test was valid, as the positive controls induced at least the minimum number of revertants as specified by the protocol. The test material was toxic at the 4 highest concentrations tested in Salmonella strain TA1537 in the first test, but not the second.
- Test condition** : Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537 were obtained from Dr. Bruce Ames (University of California, Berkeley, USA) and Escherichia coli WP2uvrA was obtained from Dr. C. Voogd, National Institute of Public Health and Environmental Protection, Bilthoven, the Netherlands. Frozen stocks of each strain were tested for histidine or tryptophan requirement and sensitivity to ampicillin, crystal violet and UV radiation (where appropriate).
- S-9 was prepared from the livers of twelve male Wistar rats five days after they had been induced i.p. with a single dose of 500 mg/kg Aroclor 1254 in soya bean oil (20% w/v). The S-9 was assayed for sterility (0 colonies/10 microliters), protein content (32.1 g/l) and cytochrome P-450 content (29.6 micromoles/l; 0.924 micromoles/g protein). S-9 was frozen until use. On the day of use, aliquots of S-9 were thawed, mixed with a NADPH-generating system, and kept on ice until use.
- The material was tested in two separate plate incorporation assays. Test material was dissolved in DMSO at 50 mg/l for the first assay and 20 mg/l for the second. A clear solution was obtained. This solution was used to make serial dilutions by 3-fold intervals in the first assay (62, 185, 556,

1667 and 500 micrograms/plate), and 2-fold intervals in the second assay (125, 250, 500, 1000, and 2000 micrograms/plate). In both assays, the 3 highest concentrations precipitated in the top agar. Positive controls were 1.0 microgram/plate sodium azide (strains TA100 and TA1535 without S-9), 80 micrograms/plate 9-aminoacridine (strain TA1537 without S-9), 2 micrograms/plate 2-nitrofluorene (strain TA98 without S-9), 100 micrograms/plate N-ethyl-N-nitrosourea (strain WP2uvrA without S-9), 2-aminoanthracene (2 micrograms/plate for strains TA98, TA100, TA1535 and 80 micrograms/plate for WP2uvrA), and bezo(a)pyrene (4 micrograms/plate for strain TA1537).

Bacteria (0.1 ml of a fully grown culture), test material, negative control (DMSO) or positive control (0.1 ml of the appropriate dilution), and 0.5 ml of sodium phosphate buffer (for tests without metabolic activation) or 0.5 ml of S-9 mix (for tests with metabolic activation) were mixed with 2 ml molten top agar (containing 0.6% agar, 0.5% NaCl and 0.05 mM L-histidine HCl/0.05 mM biotin for Salmonella and supplemented with 0.05 mM tryptophan for E coli), and the mix was poured onto minimal glucose agar plates (1.5% agar in Vogel and Bonner medium E with 2% glucose). All plates were prepared in triplicate. The plates were incubated at 37 degrees C for 3 days. Subsequently, the his<sup>+</sup> (Salmonella) and trp<sup>+</sup> (E. coli) revertants were counted. The background lawn of bacteria growth was examined microscopically to determine if the material caused toxicity.

The study was considered valid if the mean colony counts of controls were within acceptable ranges and the positive controls caused a minimum 3-fold (strains TA100 or WP2uvrA without S-9 and TA98, TA100 and TA1537 with S-9), 5-fold (strain TA1535 with and without S-9 and WP2uvrA with S-9), or 10-fold (strain TA1537 without S-9) increase in the number of revertants. In addition, plates lost through contamination or other unforeseen events had to be <= 5%.

A response was considered positive if the mean number of revertants on the test plates was 2-fold greater than that of negative controls. A response was equivocal if the mean number of revertants was increased by 2-fold in strain TA100 or slightly less than 2-fold for the other strains.

A second test was to be conducted if the first test was inconclusive. The first test was considered to be inconclusive if < 5 analyzable concentrations were obtained, if a positive or equivocal response at only one concentration was observed or if positive or equivocal responses at several concentrations were observed that were not concentration-dependent.

A test material was considered to be mutagenic if a concentration-related increase or reproducible positive response was observed. A test material was not mutagenic if it produced neither a dose-related increase in the mean number of revertants nor a reproducible response at any of the time points. Both numerical significance and biological relevance were considered together in the evaluation.

<b>Test substance</b>	:	The purity of the test material was 93.7-98.9%.
<b>Reliability</b>	:	(1) valid without restriction
<b>Flag</b>	:	The test was performed according to an established guideline.
20.05.2003	:	Critical study for SIDS endpoint
		(17)
<b>Type</b>	:	Mouse lymphoma assay
<b>System of testing</b>	:	cultured mouse lymphoma L5178Y cells
<b>Test concentration</b>	:	6.2, 12.5, 25, 50, 100 and 200 micrograms/ml for test 1, 6.6, 8.2, 10, 13, 16, 20 and 25 micrograms/ml for test 2 without S-9 and 25, 33, 41, 51, 64, 80 and 100 micrograms/ml for test 2 with S-9
<b>Cytotoxic concentr.</b>	:	> = 20 micrograms/ml (without S-9), > = 51 micrograms/ml (with S-9)
<b>Metabolic activation</b>	:	with and without

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**Result** : negative  
**Method** : OECD Test Guideline 476  
**Year** : 2001  
**GLP** : yes  
**Test substance** : as prescribed by 1.1 - 1.4

**Remark** : Turbidity was present at 100 and 200 micrograms/ml in the first experiment (both in the presence and absence of S-9). Slight turbidity was observed at 100 micrograms/ml in the second experiment (in the presence of S-9).

Although the methods section stated that 0.1 mM methyl methanesulphonate (MMS) was used as a positive control in both tests without S-9, the results table for test 1 indicates that 10 mM MMS was used. It is believed that this is a misprint, since the number of mutants produced was similar to that produced in the second study with 0.1 mM MMS.

**Result** : There was no concentration-related increase in mutants in the presence or absence of S-9 in either study. In the presence of S-9 mix, the mutant frequencies were increased at single concentrations of 100 micrograms/ml in the first test (414 x 10E6 mutants in treated vs. 889 and 109 x 10E6 mutants in the negative controls) and 33 micrograms/ml in the second (724 x 10E6 mutants in treated vs. 96 and 130 x 10E6 mutants in the negative controls). Both findings were not statistically significant. At these concentrations, more large (approximately 70%) than small colonies (approximately 30%) were formed. In the negative and positive controls incubated with S-9, the ratios of large:small colonies are approximately 60:40 and 50:50, respectively. In both assays, a Bartlett test was significant at  $p < 0.05$ , indicating the presence of an outlier. Therefore, the findings were not considered to be indicative of mutagenic activity.

In the absence of S-9 mix, a dose related decrease of the initial cell yield, relative suspension growth (RSG) and relative total growth (RTG) was observed at concentrations  $> 16$  micrograms/ml. At the highest concentration tested (25 micrograms/ml), the RTG in the first assay was 6% (only one culture survived) and 36 and 75% in the second test. In the presence of S-9 mix, the RSG and RTG were affected at concentrations  $> 41$  micrograms/ml. At the highest concentration tested in the first test (100 micrograms/ml), the RTG was 2 and 6%. In the second assay, the RTG was 31 at 26% at 80 micrograms/ml.

**Test condition** : The test was valid, since all requirements for validity were fulfilled. Mouse lymphoma L5178 cells (L5178Y tk +/- 3.2.7.c line) were obtained from Dr. J Cole, MRC Cell Mutation Unit, University of Sussex, UK. The chromosome number was 40 (stable aneuploid karyotype,  $2n = 40$ ). The cells were stored frozen in liquid nitrogen. Each new stock tested negative for mycoplasma contamination. Subcultures were prepared 5-7 days prior to experimental use. These cells (about  $1 \times 10^7/75$  cm<sup>2</sup> culture flask) were seeded in 50 ml RPMI 1640 medium (with HEPES and Glutamax-I) supplemented with 10% heat-inactivated horse serum, sodium pyruvate and penicillin/streptomycin. Cells were then incubated at 37 degrees C in humidified air containing 5% CO<sub>2</sub>. Fresh cells were harvested from a number of culture flasks and suspended in culture medium with 10% horse serum and counted. The growth rate (14.0 and 11.4 hr doubling time for tests 1 and 2) and viability of the cells (95% and 93% for each test) were checked on the day of exposure.

The test material was dissolved in DMSO (20 or 10 mg/ml for tests 1 and 2, respectively). Serial dilutions in DMSO were prepared from this clear stock solution, so that addition of 100 microliters of each to a final volume of 10 ml culture medium would produce the desired test concentrations (6.2, 12.5, 25, 50, 100 and 200 micrograms/ml for test 1, 6.6, 8.2, 10, 13, 16, 20

and 25 micrograms/ml for test 2 without S-9 and 25, 33, 41, 51, 64, 80 and 100 micrograms/ml for test 2 with S-9).

S-9 was prepared from the livers of twelve male Wistar rats five days after they had been induced i.p. with a single dose of 500 mg/kg Aroclor 1254 in soya bean oil (20% w/v). The S-9 was assayed for sterility (4 colonies/10 microliters), protein content (32.1 g/l) and cytochrome P-450 content (29.6 micromoles/l; 0.92 micromoles/g protein). S-9 was frozen until use. On the day of use, aliquots of S-9 were thawed, mixed with a NADPH-generating system, and kept on ice until use.

For the test without metabolic activation, 100 microliters of test material, negative control (1% DMSO) or positive control (0.1 mM methane methylsulphonate) and 4.9 ml culture medium (without horse serum) were added to  $5 \times 10^6$  cells in 5 ml culture medium (with 10% horse serum) to a final volume of 10 ml. Duplicate cultures for each concentration and negative control and one positive control culture were prepared. Cells were incubated for 24 hr at 37 degrees C. At the beginning and end of the study, all cultures were checked visually and selected cultures were checked for viability using trypan blue exclusion. For the test with metabolic activation, 100 microliters of test material, negative control (1% DMSO) or positive control (10 micrograms/ml 3-methylcholanthrene), 3.9 ml culture medium (without horse serum), and 1 ml 20% (v/v) S9-mix were added to  $5 \times 10^6$  cells in 5 ml culture medium (with 10% horse serum) to a final volume of 10 ml. Duplicate cultures for each concentration and negative control and one positive control culture were prepared. They were incubated for 4 hours and checked for viability as described above.

The cytotoxicity of the test material was determined by counting the cells after exposure (see above) and by measuring the relative suspension growth (cumulative growth rate of cells 24 and 48 h after treatment compared with untreated controls) and relative total growth (product of the relative suspension growth and the cloning efficiency at 48 hours).

The frequency of trifluorothymidine (TFT) mutants and cloning efficiency (see below) of the cells were determined 48 hours after treatment. At this time, the cell suspensions were diluted to 10,000/ml in culture medium (with 20% horse serum) containing 4 micrograms TFT/ml. Aliquots (200 microliters) were transferred to each well of two 96-well microtiter plates, and the plates were incubated for 10-14 days at 37 degrees C. Four plates were prepared if a reduced cloning efficiency (< 50%) was expected. After the incubation period, the number of wells containing no growth was determined and the cloning efficiency in the TFT plates was calculated. The mutant frequency/ $1 \times 10^6$  clonable cells was then calculated. The numbers of colonies that were small (which are produced predominantly by chromosome rearrangements) and large (which are produced predominantly by point mutations) in the negative and positive controls and cultures treated with some of the test concentrations also were scored.

The cloning efficiency of the cells was calculated from the total number of negative wells on two microtiter plates and the number of cells seeded per well. To assess the cytotoxic effects of the test material or the positive controls on the cells, the initial cell yield after treatment, the relative suspension growth (at 24 and 48 hours) and the relative total growth (at 48 hours) compared to that of the negative vehicle controls were calculated. The cloning efficiencies of the cells with and without TFT were used together to calculate the mutant frequency, which was expressed as the number of TFT-resistant mutants per  $1 \times 10^6$  clonable cells.

The tests were considered to be valid if the average cloning efficiency of the negative controls was  $\geq 60\%$  and  $\leq 140\%$  and fell within the range of 40-300 TFT-resistant mutants per  $1 \times 10^6$  clonable cells, the mutant

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frequency of the positive controls was > 400 mutants/ 1 x 10E6 clonable cells and was at least twice that of the negative control, and the highest concentration used should cause cytotoxicity (unless the highest possible concentration that could be tested based on solubility, pH and osmolar effects was not cytotoxic).

Results at single concentrations were compared to values for negative controls using Dunnett's multiple comparison test. Concentration-related increases in mutant frequency were determined by linear regression and lack of fit. A response was considered to be positive if the p-value of the comparison between test and negative control values was < 0.05. Any apparent increase in mutant frequency at concentrations causing > 90% cytotoxicity was considered to be an artifact. A test material was considered mutagenic if a statistically significant concentration-related increase in the frequency of mutants occurred, or if a reproducible and statistically significant positive response for at least one of the test substance concentrations was observed. A material tested negative if it produced neither a statistically significant dose-related increase in the frequency of mutants nor a statistically significant and reproducible positive response at any of the time points. Both numerical significance and biological relevance were considered together in the evaluation.

<b>Test substance</b>	:	The purity of the test material was 93.7-98.9%.
<b>Reliability</b>	:	(1) valid without restriction
		The test was performed according to an established guideline.
20.05.2003		(22)
<b>Type</b>	:	Chromosomal aberration test
<b>System of testing</b>	:	Cultured Chinese Hamster Ovary Cells (CHO K-1 line)
<b>Test concentration</b>	:	0.06 to 250 micrograms/ml (test 1); 12.5, 25, 50, 75, 125, and 250 micrograms/ml (test 2)
<b>Cytotoxic concentr.</b>	:	
<b>Metabolic activation</b>	:	with and without
<b>Result</b>	:	negative
<b>Method</b>	:	OECD Test Guideline 473
<b>Year</b>	:	1991
<b>GLP</b>	:	yes
<b>Test substance</b>	:	as prescribed by 1.1 - 1.4
<b>Remark</b>	:	For test 1 in the presence of S-9, the highest concentration tested (250 micrograms/ml) did not decrease the mitotic index. This concentration also caused a 36% decrease in the mitotic index after 4 hrs of incubation without S-9 (test 1), and 27% and 8% decreases in two experiments with S-9 (test 2). In these instances, this concentration did not cause the desired degree of inhibition (50-70%). Higher concentrations than 250 micrograms/ml could not be tested due to problems with solubility.
<b>Result</b>	:	In both assays, there was no significant effect of the test material on the number of cells with aberrations (with and without metabolic activation). In all experiments, the maximum percentage of cells treated with test material or vehicle that exhibited chromosome aberrations was 1.0%. In both assays, the negative control values were within historical ranges and the positive controls caused a significant increase in the number of cells with aberrations.
<b>Test condition</b>	:	The CHO cells (CHO K-1 line) were obtained from Dr. A. T. Natarajan, University of Leiden, The Netherlands. The chromosome number was 21-22 (stable aneuploid karyotype), and the cell cycle time was 12-14 hr. The cells were stored as frozen stock cultures in liquid nitrogen. Subcultures were prepared (passage 16) for experimental use. Cells were incubated at 37 degrees C in humidified air containing 5% CO2. At each passage, tests for mycoplasma contamination and karyotype stability were negative and stable, respectively. Cells were cultured in Ham's F-12 medium with Glutamax-I, supplemented with heat-inactivated fetal calf serum (10%), penicillin (100 IU/ml) and streptomycin (100 micrograms/ml).

S-9 was prepared from the livers of twelve male Wistar rats five days after they had been induced i.p. with a single dose of 500 mg/kg Aroclor 1254 in soya bean oil (20% w/v). The S-9 was assayed for sterility (4 colonies/10 microliters), protein content (32.1 g/l) and cytochrome P-450 content (29.6 micromoles/l; 0.92 micromoles/g protein). S-9 was frozen until use. On the day of use, aliquots of S-9 were thawed, mixed with a NADPH-generating system, and kept on ice until use.

The test material could not be dissolved in DMSO at 500 gm/ml (the concentration needed to yield the maximum concentration of 5 mg/ml stipulated by the protocol). The maximum concentration that could be dissolved in DMSO was 100 mg/ml. This concentration was sonicated for 3 min and was clear immediately after sonication (but not 30 minutes later). Serial dilutions from 0.2 - 100 mg/ml were prepared in DMSO, and 50 microliters of these solutions were added to culture medium (4.95 ml) to produce test concentrations ranging from 20 - 1000 micrograms/ml. Immediately after addition of the test material and throughout incubation at 37 degrees C for 18-24 hours, the medium was turbid and the test material flocculated in the medium at 125 - 1000 micrograms/ml. Therefore, for the first test, the highest concentration chosen was 250 micrograms/ml.

For the first test, exponentially growing cells (120,000 cells/flask) were seeded in 25 cm<sup>2</sup> tissue culture flasks containing 5 ml culture medium and incubated at 37 degrees C overnight. Cells were exposed to S-9 mix (0.5 ml) or an equivalent amount of culture medium, plus test (0.06, 0.125, 0.25, 0.5, 1, 2, 3.9, 7.8, 15.6, 31.3, 62.5, 125, and 250 micrograms/ml, negative control (DMSO) or positive control materials (0.1 and 0.025 micrograms/ml mitomycin C in the absence of S-9 and 3.75 micrograms/ml cyclophosphamide in the presence of S-9 mix) the next day. All treatments were done in duplicate. For the experiments with S-9, the culture medium did not contain fetal calf serum. The treatment time was 4 hr (pulse treatment) and 18 hr (continuous treatment) in the absence of S-9, and 4 hr (pulse treatment) and 4 hr only in the presence of S-9. Cells treated for only 4 hours were washed twice with phosphate-buffered saline and placed in fresh medium that did not contain test material after 4 hours. In both the absence and presence of S-9, the harvest time was 18 hr after treatment.

Cells were examined visually immediately after treatment, before the medium replacement at 4 hours, and 16 hours after treatment.

The conditions of the second test were identical to the first test with the following exceptions: the concentrations used were 12.5, 25, 50, 75, 125, and 250 micrograms/ml, the treatment/harvest times were 18/18 and 32/32 hr in the absence of S-9 mix and 4/18 and 4/32 hr in the presence of S-9 mix, and the positive control in the absence of S-9 mix was 0.05 micrograms/ml mitomycin C.

Two hours before incubations were terminated, cells were arrested in metaphase by adding colcemid (final concentration 0.1 mM). Cells were harvested at their designated time by trypsinization, treated for 15 min at 37 degrees C with a hypotonic solution (1% sodium citrate), fixed with a 3:1 mixture of methanol:glacial acetic acid, and transferred to slides (2/culture). Slides were stained in a 2% solution of Giemsa, rinsed in water, dried and covered with a coverslip. The slides were coded blindly. At least 1000 nuclei per culture were examined (500 per slide) to determine the mitotic index. In both assays, at the sampling times of 18 and 32 hours, the positive and negative controls and at least 3 concentrations of test material were selected for analysis of chromosomal aberrations [62.5, 125 and 250 micrograms/ml for test 1 with S-9 and the 4-hr test 1 without S-9; 15.6, 31.3 and 62.5 micrograms/ml for the 18-hr test 1 without S-9; 5, 10, and 20 micrograms/ml for test 2 without S-9 (18 and 32 hr); and 75, 125 and 250

micrograms/ml for test 2 with S-9 (both harvest times)]. If possible, the highest concentration analyzed reduced the mitotic index by 50-70% (compared to the negative control) or exhibited some other clear indication of toxicity.

For each treatment group, 200 well-spread metaphases per concentration (100 per culture), each containing 20-22 centromeres were analyzed by microscopic examination for chromatid-type aberrations (gaps, breaks, fragments, interchanges), chromosome-type aberrations (gaps, breaks, minutes, rings, dicentric) and other anomalies, such as interstitial deletions, endoreduplication, polyploidy and multiple aberrations (> 10/cell, excluding gaps), according to the criteria recommended by Savage (J Med Genet 13:103-122). Heavily damaged or endoreduplicated cells were recorded but not counted and included in the 200 analyzed cells. Gaps (achromatic lesions) were recorded separately and were not indicated in the final assessment). The Vernier readings of all aberrant metaphases were recorded.

Data from treated and control cultures were analyzed statistically by the Fisher's exact probability test (2-sided). The study was considered valid if the positive controls gave a statistically significant increase in the number of aberrant cells and the negative controls were within the historical range.

A response was considered positive if the percentage of cells with structural chromosomal aberrations is statistically higher ( $p < 0.05$ ) compared to the negative control. It was equivocal if the  $p$  value was  $> 0.05$  but  $< 0.1$ . The material was considered to be clastogenic if a dose-related increase in the percentage of cells with structural aberrations was observed compared to control, or if a single positive result occurred in both tests. A test material was negative if there was neither a dose-dependent increase in aberrations nor a reproducible positive value in the tests. Both statistical significance and biological relevance were considered together in the evaluation.

**Test substance** : The purity of the test material was 93.7-98.9%.

**Reliability** : (1) valid without restriction  
The test was performed according to an established guideline.

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## 5.6 GENETIC TOXICITY 'IN VIVO'

**Type** : Micronucleus assay  
**Species** : mouse  
**Sex** : male/female  
**Strain** : NMRI  
**Route of admin.** :  
**Exposure period** :  
**Doses** : 200, 1000 and 5000 mg/kg  
**Result** : negative  
**Method** : other  
**Year** : 1983  
**GLP** : yes  
**Test substance** : as prescribed by 1.1 - 1.4

**Remark** : It was not actually stated anywhere in the test that the material was given orally by gavage. However, based on the doses given this is assumed. The study is comparable to a guideline study.

**Result** : One male mouse treated with 5000 mg/kg died after the second application of test material. There was no significant difference between the numbers of micronucleated polychromatic (PCE) or normochromatic erythrocytes (NCE) in treated animals versus vehicle-treated controls. The average

**Test condition**

numbers of PCE and NCE in control animals were 1.8 and 0.95, respectively. In the three groups of treated animals, the average numbers of PCE and NCE ranged from 1.2-2.0 and 0.6-1.4, respectively. There was no effect of treatment on the ratio of PCE to NCE (ranged from 0.2-2.2 in control and 0.8-2.1 in treated). The test was valid, as there was a significant increase in the number of micronucleated erythrocytes in the positive control group (23-46 micronucleated PCE/animal and 6-14 micronucleated NCE/animal in positive controls vs. 0-3 micronucleated PCE and 0-2 micronucleated NCE/animal in the negative control).

: Sixty-eight NMRI KFM mice (34/sex, 5 weeks old, 20-39 g) were acclimated for 13 days before being used in the test. They were clinically examined by a veterinarian during this period and did not have any symptoms of disease. Mice were randomized into 3 treatment groups of 6 animals/sex (200, 1000 and 5000 mg/kg), one positive control group of 6 animals/sex (50 mg/kg cyclophosphamide) and one negative control group of 8 animals/sex [(Aqua Bidest with Tween 80(1%)]. For each dose of test material, a suspension was prepared by adding the material to Aqua Bidest containing 1% Tween 80. The suspension was homogenized and stirred with a magnetic stir bar while animals were being dosed. The test and control materials were administered by gavage on two consecutive days in a volume of 20 ml/kg. Animals received pelleted standard Kliba 343-QA mouse maintenance diet and water ad libitum before and during the test.

All animals were killed by cervical dislocation 24 hours after the last treatment (48 hours after the first treatment). Both femurs were removed from each mouse and freed of adherent tissue. Bone marrow cells were flushed out of the femurs using a needle and syringe containing 0.2 ml calf serum. Cells from both femurs were centrifuged at 1000 rpm for 5 min. Cells in the sediment were carefully mixed by aspiration in a siliconized Pasteur Pipette. A small drop of the suspension was smeared onto a slide and slides were air dried overnight. Two slides were prepared per animal. The following day, the slides were stained according to the Panoptic staining method of Pappenheim (as described in Queisser, Das Knochenmark, Georg Thieme Verlag, Stuttgart 1978, pg 12).

Slides from each animal (with the exception of 1 animal/sex from each treatment group) were blindly evaluated for the presence of micronuclei. One thousand polychromatic erythrocytes (PCE) and one thousand normochromatic erythrocytes (NCE) from each slide were screened under a microscope (at 1000x). The ratio of polychromatic to normochromatic erythrocytes based on 150 PCE and NCE per slide was calculated. The ratio of PCE/NCE was calculated and used as an index of toxicity of the test material.

Homogeneity of test results was confirmed using the Poisson Heterogeneity test. The results of the positive control tests were not included in the analysis since they were so much higher than those of the test material. The 95% confidence limits for the L parameter of the Poisson distributions were taken from Geigy tables. Since results did not appear to be dependent on dose of the material, all doses of the material per sex were analyzed globally (compared to the negative control) using regression. The test material was considered positive if the T value (one-sided) was significant.

**Test substance**

: The stated purity of the material was > 95%.

**Reliability**

: (1) valid without restriction

**Flag**

: Critical study for SIDS endpoint

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**5.7 CARCINOGENICITY**

## 5.8.1 TOXICITY TO FERTILITY

## 5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

## 5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

Type	: other:examination of reproductive organs from 90-day study
In vitro/in vivo	: In vivo
Species	: rat
Sex	: male/female
Strain	: Sprague-Dawley
Route of admin.	: oral feed
Exposure period	: 90 days
Frequency of treatm.	: continuous
Duration of test	: 90 days
Doses	: 25, 100 and 400 mg/kg bw
Control group	: yes
Result	: test material was not toxic to reproductive organs at the doses tested
Method	: other
Year	: 1977
GLP	: no
Test substance	: as prescribed by 1.1 - 1.4

**Remark** : This study is described in more detail in Section 5.4.

**Result** : There was no effect of treatment on any of the organs examined.

**Test condition** : COBS Sprague-Dawley rats (80 per sex) were acclimated for 8 days before treatment. They were then assigned to 4 test groups [0 mg/kg bw (control diet), or 25, 100 or 400 mg/kg bw test material] of 20 per sex per group by weight stratification. At time of assignment, all animals were 4 weeks old. Weights of male and female animals ranged from 69-100 g and 64-95 g, respectively. Animals were individually housed in suspended stainless steel cages and were maintained in a room that was kept at 70-75 degrees F and 40-50% relative humidity, with 10 exchanges of air/hour. The light cycle was 12 hr light/12 hours dark.

Test diets were prepared by mixing the appropriate amount of test material with equal amounts of ground Purina Lab Chow in a high speed blender. After blending, an additional 2 parts of ground lab chow was mixed by hand into the blended material. After this step, an additional amount of ground lab chow was added to give the appropriate dose level (25 mg/kg bw, 100 mg/kg bw or 400 mg/kg bw). The diet was then placed in a twin-shell mixer and mixed until the test material was distributed throughout the diet. The formula used to determine the amount of material added to feed was as follows: mean body weight x dietary level x 7 days/mean weekly food consumption = mg test material/kg feed. Fresh diets were prepared at least once per week. Dosages were adjusted weekly on the basis of the weight and food consumption for the preceding week.

Animals were maintained on their respective diets for a period of 90 days. As mentioned above, food consumption and body weights were measured weekly. Clinical chemistries, hematologies and urinalyses were performed on selected animals. Organ weights (brain, gonads, heart, kidneys and liver) were measured in all animals that survived to day 90.

At study termination, all animals were weighed. They were then euthanized. The urogenital orifices were examined visually and by palpation for distortions. All subcutaneous tissues were examined

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including mammary glands. Abdominal contents were examined grossly. The following reproductive tissues were taken and preserved for possible histologic evaluation: prostate, testes, uterus, ovary and seminal vesicle. Any organ or tissue exhibiting a lesion was noted and the lesion was taken for histopathological examination. Complete histopathology was performed on 10 animals/sex/group from the control and high dose groups. These animals were selected randomly from a table.

**Test substance** : The purity of the test material was 90.0%. Impurities were not listed.

**Reliability** : (2) valid with restrictions  
Doses of test material, homogeneous distribution, and stability in the diet were not confirmed analytically. The effects on mating and offspring were not tested.

**Flag** : Critical study for SIDS endpoint (20)  
21.05.2003

**Type** : other: examination of organs from 90 day study

**In vitro/in vivo** : In vivo

**Species** : dog

**Sex** : male/female

**Strain** : Beagle

**Route of admin.** : oral feed

**Exposure period** : 91 days

**Frequency of treatm.** : 1 hr/day

**Duration of test** : 91 days

**Doses** : 46 mg/kg/day

**Control group** : yes, concurrent no treatment

**Result** : no effect on the reproductive organs examined

**Method** : other

**Year** : 1983

**GLP** : yes

**Test substance** : as prescribed by 1.1 - 1.4

**Remark** : Additional information from this study is located in Section 5.4.

**Result** : There was no effect of treatment on ovary or testes weights. There also was no effect of treatment on histology of any reproductive organ examined.

**Test condition** : Purebred beagle dogs (31/sex, approximately 5 months old) were acclimated for 55 days before use. The supplier had immunized the dogs against canine distemper, infectious canine hepatitis, canine leptospirosis, parovirus and rabies. A veterinarian concluded that they were healthy prior to shipment. The body weight range of the animals just prior to treatment was 5.6 to 9.3 kg for males and 4.5 to 8.5 kg for females. The dogs were housed individually in a room maintained under a 12 hr light/12 hr dark cycle and 12 air changes/hour.

During the acclimation period, the animals were randomly assigned to 7 treatment groups of 4 animals/sex. Five of these groups were going to be treated with a compound B (which was not identified). One group served as a control group, and the other was to be treated with CL226,034 (Cyanox 1790).

Diets containing the test material were prepared weekly by adding the required weight of the test material to known amounts of powdered certified dog chow and mixing for approximately 30 minutes in a Hobart mixer. Homogeneity and adequacy of mixing of the test diets were evaluated by analyzing samples from the top, middle and bottom of the mixer. Stability of the test diet was analyzed immediately after preparation and storage at ambient temperature for 7 and 14 days. Fresh diets were stored at approximately 4 degrees C in labeled airtight plastic containers for the period of use. The concentration of test material in the diets was checked during weeks 1, 2, 4, 8 and 13, immediately after preparation.

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From study day -5.1 to 0.1, the dogs were fed 400 g/day of a standard certified commercial dog food. From study day 0.2 to termination, the males were fed 450 g/day and the females 400 g/day. For one hour per day, test animals were fed diet containing test material (compound B or 46 mg/kg/day CL226,034). Fresh water was available ad libitum. There were no contaminants in the food or water that were expected to affect the outcome of the test. All controls and animals treated with CL226,034 were on test for a minimum of 91 days.

A complete gross necropsy was performed on each dog that survived to study termination. The animals were euthanized and exsanguinated, and the ovaries and testes were dissected and weighed. The ovaries, testes, epididymides, prostate, uterus (horns and body), and vagina were examined histologically.

**Test substance** : The test material was CL 226, 034, which is Cyanox 1790. It was supplied as a white powder from American Cyanamid. Documentation of purity did not accompany the study. According to a Cytec Industries Inc. (formerly known as American Cyanamid) MSDS of the material written in 2001, the purity of the material is 93.7 - 98.9%. Impurities are not listed on the MSDS.

**Reliability** : (2) valid with restrictions  
The effects on mating and offspring were not tested. Only one dose was tested.

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**Type** : other:examination of organs from 90 day study  
**In vitro/in vivo** : In vivo  
**Species** : dog  
**Sex** : male/female  
**Strain** : Beagle  
**Route of admin.** : oral feed  
**Exposure period** : 90 days  
**Frequency of treatm.** : continuous  
**Duration of test** : 90 days  
**Doses** : 7.5, 15, 30 mg/kg bw  
**Control group** : yes, concurrent no treatment  
**Result** : not toxic to the reproductive organs examined  
**Method** : other  
**Year** : 1977  
**GLP** : no  
**Test substance** : as prescribed by 1.1 - 1.4

**Remark** : This study is described in more detail in Section 5.4.  
**Result** : There was no effect of treatment on the gross or histopathology of any organ examined.

**Test condition** : Thirty two pure bred Beagle dogs (16 per sex) were acclimated for 16 days before treatment. They were vaccinated against canine distemper, hepatitis and leptospirosis by the supplier. Animals were individually housed in suspended stainless steel cages and were maintained in a room that was kept at 70-76 degrees F and 25 - 50% relative humidity. The light cycle was 12 hr light/12 hours dark. Cages were cleaned semi-monthly.

Dogs were assigned to 4 test groups [0 mg/kg bw (control diet), or 7.5, 15 or 30 mg/kg bw test material] of 4 per sex per group one week prior to treatment by weight stratification. On day 1 of treatment, weights and ages of the animals were 5.1 - 6.7 kg and 4 - 5 months, respectively.

Test diets were prepared by geometrically diluting the appropriate amount of test material with Purina Laboratory Canine Diet in a grounded twin shell blender. Diets containing the least amount of test material were mixed first. The blender was completely cleaned between mixing each dose level. The formula used to determine the amount of material added to feed was as

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follows: mean body weight (g) x dose (mg/kg bw)/mean food consumption per day (g) = mg test material/kg diet. The desired doses were 7.5, 15.0 and 30.0 mg/kg bw. Doses were not adjusted for purity of the material. Doses were adjusted weekly based on the group body weights of dogs for that week. Diets were prepared weekly.

Animals were maintained on their respective diets for a period of 90 days. As mentioned above, food consumption and body weights were measured weekly. At study termination (days 90 and 9a), all animals were weighed. They were then euthanized and exsanguinated. The gonads were weighed. Small portions of the following reproductive tissues were taken and preserved in 10% neutrally buffered formalin for possible histologic evaluation: ovary, prostate, testis, and uterus. Sections were prepared at 5 microns from paraffin blocks and stained with hematoxylin and eosin.

Data for organ weights were analyzed using a randomized block analysis of variance. The level of significance is  $p < 0.05$ . No statistical evaluations were performed on incidences of gross or microscopic lesions.

- Test substance** : The page describing the purity of the test material was missing from the report. However, the purity of the test material used in a rat study that was performed at the same facility around the same time was 90.0%. Impurities were not listed.
- Reliability** : (2) valid with restrictions  
Doses of test material, homogeneous distribution, and stability in the diet were not confirmed analytically. The effects on mating and offspring were not tested.

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### 5.9 SPECIFIC INVESTIGATIONS

### 5.10 EXPOSURE EXPERIENCE

### 5.11 ADDITIONAL REMARKS

**6.1 ANALYTICAL METHODS**

**6.2 DETECTION AND IDENTIFICATION**

**7.1 FUNCTION**

**7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED**

**7.3 ORGANISMS TO BE PROTECTED**

**7.4 USER**

**7.5 RESISTANCE**

**8.1 METHODS HANDLING AND STORING**

**8.2 FIRE GUIDANCE**

**8.3 EMERGENCY MEASURES**

**8.4 POSSIB. OF RENDERING SUBST. HARMLESS**

**8.5 WASTE MANAGEMENT**

**8.6 SIDE-EFFECTS DETECTION**

**8.7 SUBSTANCE REGISTERED AS DANGEROUS FOR GROUND WATER**

**8.8 REACTIVITY TOWARDS CONTAINER MATERIAL**

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### 10.1 END POINT SUMMARY

### 10.2 HAZARD SUMMARY

### 10.3 RISK ASSESSMENT