AR201-13271B

BOILING POINT

Test Substance: Diethylene glycol dibenzoate is a pale colored liquid.

Purity Profile:

NAME	CAS #	%
Diethylene Glycol Dibenzoate	120-55-8	**89.9**
Diethylene Glycol Monobenzoate	20587-61-5	5.85
Dipropylene Glycol Dibenzoate	27138-31-4	1.50
Ethylene Glycol Dibenzoate	94-49-5	0.19
Triethylene Glycol Dibenzoate	120-56-g	0.15
Unknown 1	unknown	0.16
Unknown 2	unknown	0.10
Unknown 3	unknown	0.54

Test Method: OECD 103, EEC A2

GLP: **Yes**

Year Performed: 1997

Results: Decomposes @ > 230°C without boiling

Data Quality: 1, Reliable without restrictions

References: Benzoflex 2-45 Determination of Physical/Chemical Properties Report. Huntingdon Life Sciences. 1999

FREEZING POINT

Test Substance: See boiling point for purity

Test Method: OECD 102, EEC Al

GLP: **Yes**

Year Performed: 1997

Results: Freezing point 24°C

Data Quality: 1 – Reliable without restrictions

References: Benzoflex 2-45 Determination of Physical/Chemical Properties Report. Huntingdon Life Sciences. 1999

VAPOR PRESSURE

Test Substance: See boiling point for purity

Test Method: OECD 104, EEC A4

GLP: **Yes**

Year Performed: 1997

Results: 1.3×10^{-7} mm Hg @ 25°C
3.2×10^{-6} mm Hg @ 50°C
5.1×10^{-4} mm Hg @ 100°C

Data Quality: 1, Reliable without restrictions

References: Benzoflex 2-45 Determination of Physical/Chemical Properties Report. Huntingdon Life Sciences. 1999

PARTITION COEFFICIENT

Test Substance: See boiling point for purity

Test Method: OECD 107 & 117, EEC A8

GLP: **Yes**

Year Performed: 1997

Results: log P_{ow} = 3.2

Calculated Value: 3.0406

Measured Value: 3.2

Data Quality: 1, Reliable without restrictions

References: Benzoflex 2-45 Determination of Physical/Chemical Properties Report, Huntingdon Life Sciences. 1999

WATER **SOLUBILITY**

Test Substance: See boiling point for purity

Test Method: OECD 105, EEC A6

GLP: **Yes**

Year Performed: 1997

Results: 38.3 mg/l @ 30°C, pH = 7.0 Low solubility

Data Quality: 1, Reliable without restrictions

References: Benzoflex 2-45 Determination of Physical/Chemical Properties Report, Huntingdon Life Sciences. 1999

ENVIRONMENTAL FATE

PHOTODEGRADATION

The rate constant and half-life for the atmospheric gas-phase reaction between photolytically produced hydroxyl radicals and diethylene glycol dibenzoate have been estimated using the software AOPWIN VI .88.

The calculated rate constant for the reaction, assuming a 24-hour day and a hydroxyl radical concentration of 1.5 x 1 0^{6} mol/cm^{3} was 18.95 x 10^{-12} cm^{3}/mol-sec and the half-life was 0.282 days (6.772 hours).

The program did not estimate a rate constant for reaction with ozone (only olefins and acetylenes are estimated) and there were no structures matched with the experimental database.

Reliability: Estimated value based on accepted model.

Reference: Diethylene Glycol Dibenzoate. Estimation of Photodegradation Using the Atmospheric Oxidation Program (AOPWIN). Huntingdon Life Sciences. 2001.

STABILITY IN WATER

The aqueous hydrolysis rate constant and half-life for diethylene glycol dibenzoate have been estimated using the software program HYDROWIN v1 .66.

The calculated rate constant for the base-catalyzed reaction (K_b) at pH>8 and at 25°C was estimated to be 1.645 x 10^{-1} Umol-sec. Half-lives at pH 7 and 8 were estimated to be 1.335 years and 48.77 days, respectively.

The program does not calculate neutral hydrolysis rate constants. Therefore, reported half-lives may be overestimates.

The fragment –CH_2- CH_2-O-R was not available in the program's library. Therefore, fragment - CH_2-CH_2–0-CH_3 was substituted. This is unlikely to have affected the estimation result significantly.

Reliability: Estimated value based on accepted model.

References: Diethylene Glycol Dibenzoate. Estimation of Hydrolysis Rate Using the HYDROWIN Program. Huntingdon Life Sciences. 2001.

TRANSPORT **(FUGACITY)**

The fate and behavior of diethylene glycol dibenzoate in a model environment consisting of four main components, air, water, soil and sediment, has been evaluated using the Mackay Level III Fugacity Model, version 2.10.

Results from the evaluation indicate that the distribution of diethylene glycol dibenzoate between the soil and water compartments is likely to be highly dependent on the route of entry, as movement between environmental compartments is very restricted except for deposition onto soil and water from air. Air concentrations will be small because of the low vapor pressure of diethylene glycol dibenzoate.

The main mechanisms of loss will be microbial degradation in soil and water and atmospheric oxidation. It appears probable that susceptibility to microbial degradation in both the soil and water compartments will result in low persistence of diethylene glycol dibenzoate.

Inputs to the Model:

Physical Chemistry Properties

Chemical Type	1	A chemical that partitions into all media
Molecular mass	314.34	Molecular formula $C_{18}H_{18}O_5$
Data Temperature	25°C	
Log K_{ow}	3.2	VCL261/972408
Water Solubility (g/m^3)	38.3	VCL261/972408
Vapor Pressure (Pa)	1.73E-05	VCL26 II972408
Melting Point	24°C	VCL 2611972408

Half-Lives

Half-life in Air	6.772 hours	VCL363/010076
Half-life in Water	62.5 hours	VCL298/983319 A factor of ten was added for this study to allow for probability of lower degradation in some other water bodies.
Half-life in Soil	125 hours	Assumed twice that of water. This is in line with advice in the TGD Part II for a readily biodegradable substance with a soil-water partition coefficient of less than 100 l/kg.
Half-life in Bulk Sediment	125 hours	Assumed twice that of water. This is in line with advice in the TGD Part II for a readily biodegradable substance with a soil-water partition coefficient of less than 100 l/kg.
Half-life in Suspended Sediment	62.5 hours	No data therefore because the close proximity of the bulk water phase the half-life for the latter was used.
Half-life in Fish	62.5 hours	No data therefore because the close proximity of the bulk water phase the half-life for the latter was used.
Half-life in Aerosol	6.772 hours	No data therefore because the close proximity of the bulk air phase the half-life for the latter was used.

Dimensions and Other Properties

The parameters that define the model environment are:
Volume of each environmental compartment (m^3)
Density of each environmental compartment (kg/m^3)
Organic carbon content of soil and sediments (g/g)
Lipid content (kg/m^3)
Transport velocities between compartments (m/h)

Emissions to the Model Environment

The route and magnitude of emissions to the environment will vary depending on the stage of the product life cycle being considered. Because no information was available on probable real-life emissions into the environment the recommendation of Mackay et al (1996) was followed; that is the model was run for 1000 kg/h emissions to each of the air, water and soil compartments individually and then in total. This standardized approach allows comparison with other compounds and provides information on the main source of the chemicals in each compartment. Specific properties which should be taken into account when evaluating outputs from the model are that it has a very low vapor pressure so that losses to the atmosphere during manufacture and processing will be limited and that its ready biodegradability means that concentrations in the effluent from sewage treatment plants will be very low.

Results

Under equilibrium steady state condition (Fugacity Model Level 1) diethylene glycol dibenzoate distributed almost entirely between the soil and water compartments

Compartment	Amount
Air	0.0012%
Soil	57.62%
Water	41.05%
Sediment	1.28%

Using the Level III program and with emissions of 1000 kg/h to each of the air, water and soil compartments, the model estimated the following distribution:

Compartment	Amount
Air	0.73%
Soil	76.1%
Water	23.2%
Sediment	0.040%

The predominant routes of loss were by degradation in the soil, water and air compartment (53.8, 32.8 and 9.50% respectively) and advection from the water compartment (2.96%). The estimated mean residence time for diethylene glycol dibenzoate in the model environment (persistence) was 128 hours.

Reliability: Estimated values based on accepted model.

References:

Diethylene Glycol Dibenzoate. Estimation of Environmental Fate Using the Mackay Level II Fugacity Model. Huntingdon Life Sciences. 2001.

VCL 261 - Benzoflex 245. Determination of Physico-Chemical Properties, Huntingdon Life Sciences. 1999

VCL363 – Diethylene Glycol Dibenzoate. Estimation of Photodegradation Using the Atmospheric Oxidation Program (AOPWIN). Huntingdon Life Sciences. 2001.

VCL 298 – Diethylene Glycol Dibenzoate. Preliminary Assessment of its Degradation in Laboratory and Natural Waters. Huntingdon Life Sciences, 1999.

Technical Guidance Document (TGD) in Support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and Commission regulation (EC) No. 1488194 on Risk Assessment for Existing Substances Part II, pp. 282-285.

Mackay, D., DiGuardo, A.., Paterson, S. and Cowan C. (1996) Evaluating the Environmental Fate of a Variety of Types of Chemicals Using the **EQC** Model. Environ Toxicol Chem, 15, p. 1627.

BIODEGRADATION

Test Substance: See boiling point for purity

Test Method: OECD 301 B, EC C. 4-E

GLP:	Yes
Test Type:	Aerobic
Year Performed:	1997
Laboratory:	Huntingdon Life Sciences
Inocculum:	Activated sludge from sewage treatment works
Concentration:	8.9 mgC/l
Duration:	29 days
Positive Control:	Sodium benzoate plus inocculum mineral salts medium
Control and Blank:	Mineral salts and medium plus inocculum
Results:	*17%* of TCO_2 @ 2days *71%* of TCO_2 @ 10 days 93% of TCO_2 @ 28 days Final mean level of biodegradation including residual CO_2 released from t he medium acidification 93%. Readily biodegradable
Data Quality:	1, Reliable without restrictions
References:	Assessment of ready biodegradability - Modified Sturm Test. Huntingdon Life Sciences. 1998

ACUTE **TOXICTY** TO FISH

Test Substance:	See boiling point for purity
Test Method:	OECD 203
Test Type:	Acute Toxicity to Fish
GLP:	**Yes**
Year Performed:	1997
Species/Strain:	Fathead minnow (*Pimephales promelas*)
Analytical Monitoring:	Every 24 hours
Exposure Period:	96 hours
Test Details:	Semi-static conditions
Statistical Methods:	LL_{50} values (median initial loading values) and 95% confidence limits were calculated using the Thompson and Weil model (Thompson and Weil, 1952).

Thompson, W.R. & Weil, C.S., 1952, Biometrics 8:51-54.

Test Condition Remarks:

Fish Size and Age: Juvenile *Pimephales promelas* mean standard length was 2.9 cm. The mean weight was 0.33 g for Replicate 1 and 0.26 g for Replicate 2.

Test Conditions: As specified in OECD 203

Diluent Water Source and Chemistry:

Laboratory tap water filtered, dechlorinated and softened by passage through an Elga® water purification system. Chlorine levels ranged form 0.03 to 0.13 mg/L throughout the 7 days of the acclimatization period and the exposure period and the hardness level, calculated from daily measurements during the same period were between 65 and 71 mg $CaCO_3$/L.

Stock and Test Solutions:

The method of preparation was selected following advice given in ECETOX 1996, Monograph No. 26. The test material is a complex mixture of components with poor but variable water solubility. In such cases it is considered that the water accommodated fraction is the most appropriate exposure medium.

The following volumes were added by accurate pipette to duplicate glass 20 L vessels of dilution water. The vessels were stirred for at least 18 hours using a magnetic stirrer with a 10 cm magnetic follower, at approximately 250 revolution / minute.

Initial Loading (mg/l)	Volume (ml)	Equivalent weight of material (g) in 20 L
37.5	0.640	0.75
7.5	0.128	0.15
1.5	0.026	0.03
0.3	0.005	0.006
0.06	0.001	0.0012

The solutions were left to stand for one hour prior to removing the water accommodated fraction by peristaltic pump. Approximately 18 L of solution was removed from a mid water position and duplicates were pooled to give the exposure solution.

Vessels and Lighting: Five test concentrations plus one control were prepared, each in duplicate. Ten fish were added to each vessel. Fish were placed at random in glass aquaria containing prepared test medium or diluent water, as appropriate. The test chambers were glass aquariums (25X46X25 cm) containing approximately 18 liters of medium to a depth of 17 cm. Supplementary aeration was provided via narrow bore glass tubes. A photoperiod of 16 hours light: 8 hours dark was maintained and daily records of temperature, pH and dissolved oxygen were kept for each control and test vessel. The fish were not fed during the 96-hour exposure period.

Fish per Vessel: 10 fish in each vessel

Dose Selection: Nominal: 0.06, 0.3, 1.5, 7.5 and 37.5 mg/L.

Renewal and Exposure: Fish were exposed to the test or control conditions for a period of 96 hours with daily batchwise renewal of the test medium to ensure the maintenance of satisfactory environmental conditions and optimal exposure levels.

Temperature range: Treatment and controls groups were maintained at 23±1°C throughout the exposure period.

Analytical Results:

Occasion	Nominal Concentration (mg/L)	Measured Concentration Dibenzoate (mg/L)		
		Tank 1	Tank 2	Mean
0 Hours (Fresh)	Control	0.02048	0.01695'	0.01872
	0.06	0.08560		
	0.3	0.2317		
	1.5	1.207		
	7.5	1.623		
	37.5	4.707		
24 Hours (Expired)	Control	0.004396	0.05604	0.03022
	0.06	0.1596	0.02716	0.09340
	0.3	0.07692	0.05210	0.06451
	1.5	0.008772	ND	
	7.5	0.7837	0.6042	0.6940
	37.5	2.224	2.324	2.274
24 Hours (Fresh)	Control	ND		
	0.06	0.08427		
	0.3	0.2326		
	1.5	1.318		
	7.5	6.547		
	37.5[2]	25.94		
48 Hours (Expired)	Control	0.01959	0.002602	0.01109
	0.06	ND	ND	
	0.3	ND	ND	
	1.5	ND	ND	
	7.5	1.002	0.8900	0.94587
	37.5	5.146	5.590	5.36785
48 Hours (Fresh)	Control	ND		
	0.06	0.1179		
	0.3	0.2006		
	1.5	1.199		
	7.5	5.507		
72 Hours (Expired)	Control	ND	ND	
	0.06	ND	ND	
	0.3	ND	ND	
	1.5	ND	ND	
	7.5	ND	ND	
72 Hours (Fresh)	Control	ND		
	0.06	0.08183		
	0.3	0.2380		
	1.5	1.185		

	7.5	5.807		
96 Hours (Expired)	Control	ND	ND	
	0.06	ND	ND	
	0.3	ND	ND	
	1.5	ND	ND	
	7.5	ND	ND	

ND – None detected (limit of detection: 0.001 mg/L)
1 Duplicate sample analyzed
2 Duplicate sample analyzed due to minor modification of method

Results:

Time (hours)	LL_{50} (mg/L)	95% confidence limits (mg/L)
3	> 37.5	----
24	> 37.5	----
48	6.4	4.4 – 9.4
72	4.3	3.1 – 5.8
96	3.9	3.0 – 5.2

Highest loading rate resulting in 0% mortality: 1.5 mg/L
Lowest loading rate resulting in 100% mortality: 37.5 mg/L
"No-observed effect loading rate": 1.5 mg/L *

* 5% mortalities were observed in the 0.03 and 0.6 mg/L levels after 96 hours. This level of mortality is not considered biologically significant, therefore the "no observed effect loading rate" is 1.5 mg/L.

The mean measured concentration of diethylene glycol dibenzoate in fresh samples was between 13 and 154% of the total loading rate. The measured amount in exposure medium was generally greater proportionally as initial loading rate decreased. In the 24 hour expired samples, the majority of the dibenzoate component had degraded. At 72 and 96 hours, none of the compound was detectable in the remaining exposure levels.

Environmental parameters (pH, T^0C, and mgO_2) remained within acceptable limits throughout the duration of the study.

Conclusions: The 96-hour LL_{50} value for Benzoflex 2-45 with fathead minnow was 3.9 mg/L. The no-observed effect loading rate for Benzoflex 2-45 with fathead minnow was 1.5 mg/l.

Data Quality: 1, Reliable without restrictions

References: Diethylene Glycol Dibenzoate. Acute Toxicity for Fathead Minnow (*Pimephales promelas*). Huntingdon Life Sciences. 1998.

ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Test Substance: See boiling point for purity

Test Method: OECD 202 Part 1

Test Type: *Daphnia* Acute Immobilization Test

GLP: **Yes**

Year Performed: *1997*

Species/Strain: *Daphnia magna*

Analytical Monitoring: Determination of test material concentration at 0 and 48 hours

Exposure Period: 48 hours

Test Details: Static without renewal

Statistical Methods: EL_{50} and 95% confidence limits were calculated using the Thompson and Weil model (Thompson WR and Weil CS, 1952, Biometrics 8: 51-54).

Test Condition Remarks:

Age at Initiation: Less than 24 hours

Test Conditions: As specified in OECD 202 following advice given in ECETOC 1996 Monograph No. 26.

Solvent: Reconstituted medium Elendt M4.

Vessel: 250 ml capacity glass jars containing 200 ml of prepared test medium. The jars were loosely covered to minimize evaporative losses.

Daphnids per Vessel: 5

Dose Selection: Nominal initial loading rate: 1.0, 2.2, 4.6, 10, 22, 46 and 100 mg/L.

Temperature range: 19-20 °C

Solution pH range: 7.5 – 7.9

Dissolved Oxygen: 7.9 - 9.0

Analytical Results:

Occasion	Nominal Initial Loading(mg/L)	% of Loading Monobenzoate (mg/L)	% of Loading Dibenzoate (mg/L)
0 Hours (Fresh)	Control	ND	ND
	1.0	8.5	54.2
	2.2	2.6	17.9
	4.6	11.8	76.1
	10	9.4	37.1
	22	9.9	60.0
	46	8.2	9.3
	100	8.9	17.8
48 Hours (expired)	Control	ND	ND
	1.0	1.9	8.7
	2.2	ND	2.7
	4.6	26.4	46.9
	10	14.3	20.1

	22	*20.0*	*60.9*
	46	*9.6*	*6.0*
	100	*8.7*	*17.7*

ND = None detected

Results:

Time (hours)	EL_{50} (mg/L)	95% confidence limits (mg/L)
24	*< 100*	----
48	*6.7*	*4.4-10.0*

Highest initial loading rate resulting in 0% mortality: < 1 .O mg/L
Lowest initial loading rate resulting in 100% mortality: > 100 mg/L
No-observed effect loading rate: 1 .0 mg/L*

*10% immobilization was observed in the 1.0 mg/L test group. This level of immobilization is not considered significant, therefore the "no-observed effect loading rate" is 1 .0 mg/L.

Nominal Initial Loading Rate (mg/L)	Cumulative immobilized *Daphnia magna* (initial population 5/replicate) 24 hours						Cumulative immobilized *Daphnia magna* (initial population S/replicate) 48 hours					
	RI	R2	R3	R4	Total	%	RI	R2	R3	R4	Total	%
Control	0	0	0	0	0	0	*1*	*1*	*0*	*0*	*2*	*10*
1.0	0	0	0	0	0	0	*0*	*1*	*1*	*0*	*2*	10
2.2	0	0	0	0	0	0	*3*	*2*	*1*	*2*	*8*	*40*
4.6	0	0	0	0	0	0	*4*	*3*	*1*	*2*	10	*50*
10	*0*	*1*	*2*	*1*	*4*	*20*	*3*	*3*	*4*	*4*	*14*	*70*
22	*0*	*1*	*1*	*2*	*4*	*20*	*5*	*3*	*4*	*5*	*17*	*85*
46	*0*	*1*	*0*	*1*	*2*	10	*4*	*4*	*4*	*4*	*16*	*80*
100	*2*	*3*	*2*	*3*	*10*	*50*	*4*	*5*	*5*	*5*	*19*	*95*

RI = Replicate 1
R2 = Replicate 2
R3 = Replicate 3
R4 = Replicate 4

Conclusions: The 48-hour EL_{50} was determined to be 6.7 mg/l. The No Observed Effect Loading rate was 1 .0 mg/l.

Data Quality: 1, Reliable without restrictions

References: Diethylene Glycol Dibenzoate. Acute Toxicity to *Daphnia magna.* Huntingdon Life Sciences. 2001.

ACUTE TOXICITY TO AQUATIC PLANTS

Test Substance: See boiling point for purity

Test Method: OECD 201

Test Type: Algae Growth Inhibition Test

GLP: **Yes**

Date Performed: 1997

Species: *Selenastrum capricornutum,* Strain number CCAP 27814

Element basis: Area under the curve (72 hours, 96 hours), growth rate (O-72 hours, O-96 hours)

Exposure period: 96 hours (December 1 - 4, 1997)

Test organisms: Sterile nutrient medium was inoculated from a master culture and cultured under continuous illumination (-7000 lux) in an orbital incubator at 22°C, to give an algal suspension in log phase growth characterized by a cell density of 4.77 xl 0^6 cells/ml.

Test Conditions:

Test Temperature Range: 24 ± 1°C

Exposure Vessel Type: 250 ml conical flask each containing 100 ml of test or control culture was loosely stoppered and placed without conscious bias, in a Gallenkamp Illuminated Orbital Incubator.

Light levels and quality during exposure: The cultures were incubated, without medium renewal for 96 hours under continuous illumination of approximately 7000 lux provided by 7X30 W "universal white" 1 meter fluorescent tubes.

Test Design:

Number of replicates: Eight exposure levels were prepared plus one untreated control, each in triplicate

Nominal Initial Loading Rates: 0.46, 1.0, 2.2, 4.6, 10, 22, 46 and 100 mg/l

Analytical Results:

Occasion	Nominal Loading Rate (mg/L)	Measured Concentration Di benzoate (mg/L)		
		Analysis 1	Analysis 2	Mean
0 Hours (Fresh)	Control	0.01658	0.02299	0.01979
	0.46	1.838	1.867	1.853
	1.0	0.4959		
	2.2	1.745		
	4.6	1.751		
	10	3.666		
	22	8.293		
	46	14.83		
	100	18.80		
	10 no algae	3.255		
96 Hours (Expired)	Control	0.02575		
	0.46	ND		
	1.0	ND		
	2.2	ND		
	4.6	ND		
	10	ND		
	22	ND		
	46	ND		
	100	ND		
	10 no algae	ND		

ND None detected (LOD: 0.0001 mg diethylene glycol dibenzoate / L

Results:

	NOEL	EL_{10}	EL_{50}	EL_{90}
Area Under curve (72 hours)	1.0	2.7	5.2	10
Growth Rate (O-72 hours)	2.2	3.3	11	37
Area Under Curve (96 hours)	2.2	3.6	5.9	9.7
Growth Rate (O-96 hours)	2.2	3.6	15	59

*A No-observed effect loading rate, 96-hour NOEL was 1 .0mg/L.

Mean cell density of control at 0 hour 1.30 x 1 0^4 cells/ml
Mean cell density of control at 96 hour 4.95 x 1 0^6 cells/ml

Chemical analyses of the two main components in fresh and expired water samples were carried out at 0 hours (fresh) and 96 hours (expired). The mean measured concentration of diethylene glycol dibenzoate in fresh samples was between 18.8 and 79.3% of the total loading rate. For diethylene glycol monobenzoate, the mean measured concentration in fresh samples was between 4.3 and 5.3% of the total loading rate. In expired samples, the majority of the two components had degraded.

Conclusions: Benzoflex 2-45 inhibited the growth of *Selenastrum capricornutum* at concentrations tested in excess of 1 .O mg/L under the conditions of this test.

The EL_{50} (Area under the curve 72 hour) was 5.2 mg/L and the EL_{50} (Growth rate 0-72 hour) was 11 mg/L, while the EL_{50} (Area under the curve 96 hour) was 5.9 mg/L and the EL_{50} (Growth rate O-96 hour) was 15 mg/L.

Data Quality: 1, Reliable without restrictions

References: Benzoflex 2-45. Algal Growth Inhibition. Huntingdon Life Sciences. 2001

ACUTE **TOXICTY**

ORAL

Test Substance: See boiling point for purity

Study Type: Acute Oral

Test Method: OECD 401

GLP: **Yes**

Year Performed: 1997

Laboratory: Huntingdon Life Sciences

Species/Strain: Rat, Sprague-Dawley

Sex: Male and Female

Results: Acute Oral LD_{50} and 95% confidence limits
Male: 4843 (4198-5588) mg/kg body weight
Female: 3535 (2892-4322) mg/kg body weight
Combined: 4190 (3504-5072) mg/kg body weight

Number of Deaths at Each Dose Level:
3200 mg/kg: 1 female
5000 mg/kg: 3 males and 5 females

Time of Death of Each Animal:

Day 2
5000 mg/kg: 1 male
5000 mg/kg: 3 females

Day 3
5000 mg/kg: 2 males
5000 mg/kg: 2 females

Day 5
3200 mg/kg 1 female

Description of Clinical Effects:

Piloerection was observed in all rats within 8 minutes of dosing. This sign persisted and was accompanied in rats later during the study by:

Hunched posture, waddling/unsteady gait, lethargy and ungroomed appearance in all rats at all dosages

Partially closed eyelids in two females at 3200 mg/kg, in all rats at 5000 mg/kg;

Pallid extremities in all females at 2000 mg/kg and all rats at 3200 and 5000 mg/kg;

Increased salivation in one male at 2000 mg/kg, four females at 3200 mg/kg, all males and four females at 5000 mg/kg;

Walking on toes in all rats at 2000 and 5000 mg/kg and all males and four females at 3200 mg/kg;

Respiratory distress (characterized by increased or decreased gasping or noisy respiration) in all rats at 2000 mg/kg, all males and four females at 3200 mg/kg and three males and all females at 5000 mg/kg;

Chronic convulsions in four males at 5000 mg/kg;

Increased lacrimation in one female at 2000 mg/kg and all males and two females at 5000 mg/kg;

Cold body surfaces in all males at 2000 mg/kg and in all rats at 3200 and 5000 mg/kg;

Prostration in one female at 3200 mg/kg and four males and two females at 5000 mg/kg;

Stained muzzle and/or urogenital area in two females at 2000 mg/kg, in all rats at 3200 mg/kg and in four males and two females at 5000 mg/kg;

Sensitivity to handling in one male and all females at 2000 mg/kg, all rats at 3200 mg/kg and four males and two females at 5000 mg/kg;

Hyperactivity in one male at 500 mg/kg;

Thin appearance in one female at 3200 mg/kg and one male at 5000 mg/kg;

Straub tail in two males at 5000 mg/kg;

Body tremors in one male and one female at 2000 mg/kg, four females at 3200 mg/kg and all males and two females at 5000 mg/kg;

Faecal disturbances (characterized by soft to liquid or lack of faeces) in all females at 2000 mg/kg and in two males at 5000 mg/kg;

Recovery of surviving rats was complete, with the exception of piloerection and unsteadiness in males at 5000 mg/kg, by either Day 10 (3200 mg/kg), Day 11 (2000 mg/kg), or Day 13 (males 5000 mg/kg).

Necropsy findings:

Females at 3200 mg/kg

Congestion (characterized by prominent blood vessels) in brain and gaseous distension in the stomach and along the alimentary tract.

Males and Females 5000 mg/kg

Congestion (characterized by dark appearance/prominent blood vessels/inflammation) in brain, heart, lungs, liver, spleen and kidneys. Congestion or pallor with fluid contents, gaseous distension and black patches were also seen in the stomach and along the alimentary tract.

Data Quality: 1, Reliable without restrictions

References: Benzoflex 2-45, Acute Oral Toxicity to the Rat, Huntingdon Life Sciences. 1998

DERMAL

Test Substance: See boiling point for purity

Study Type: Acute Dermal Toxicity

Test Method: OECD 402

GLP: Yes

Year Performed: 1997

Laboratory: Huntingdon Life Sciences

Species/Strain: Rat, Sprague-Dawley

Sex: Male and Female

Results: Acute Dermal LD_{50} >2000 mg/kg

Number of Deaths at Each Dose Level: No deaths

Description of Clinical Effects: No signs of systemic reaction to treatment were observed in any animal throughout the observation period.

Data Quality: 1, Reliable without restrictions

References: Benzoflex 2-45 Acute Dermal LD_{50}. Huntingdon Life Sciences. 1998

REPEATED DOSE TOXICITY

Test Substance: See boiling point for purity

Vehicle: Administered by dietary admixture

Study type: Dietary administration for 13 weeks with a subsequent 4-week recovery period for selected animals

Method: OECD 408

GLP: **Yes**

Year Performed: 1997

Laboratory: Huntingdon Life Sciences

Species/Strain: Crl: (IGS) CD@ BR

Age at Study Initiation: ~ 7-8 weeks

Route of Administration: Dietary

Frequency of Treatment: Continuous in diet

Duration of Test: 13 weeks with subsequent 4-week recovery for selected animals

Dose/Concentration Levels: Control, 250, 1000, 1750 OR 2500 mg/kg/day 10 male and 10 female per group

Post-Exposure Observation: An additional 10 male and 10 female rats at the control and 2500 mglkglday level served as a recovery group for a subsequent 4 weeks.

Clinical Observations Performed: Individual animals were observed and palpated at least once daily for any signs of behavioral changes, reaction to treatment or ill health. A detailed clinical observation was performed daily for the duration of the study. The weight of each rat was recorded at the time of allocation of animals to groups, on the day of commencement of treatment and once a week thereafter, including the day of death. The quantity of food consumed by each cage of rats was recorded weekly. Food conversion ratios were calculated, where possible, from the weekly bodyweight and food consumed per unit gain in bodyweight. At weekly intervals, the group mean achieved intake of test substance (mg/kg/day) was calculated from the group mean bodyweight and food consumption data as weight of food consumption and the dietary inclusion levels of the test material. Daily monitoring by visual appraisal of the water bottles was maintained throughout the study. The eyes of all rats were examined using a Keeler indirect opthalmoscope before dosing commenced. During Week 13 the eyes of all animals in Groups 1 and 5 were examined. As there was no effect of treatment, further investigations were not performed.

RESULTS

NOAEL: 1000 mg/kg/day or below are considered to represent a No Observable Adverse Effect level of Benzoflex 2-45 in rats by oral administration for 13-weeks.

Actual Dose Received: Achieved Group Mean Intakes of Benzoflex 2-45

Group 2 Males: 241 mg/kg/day
Group 3 Males: 960 mg/kg/day
Group 4 Males: 1679 mg/kg/day
Group 5 Males: 2407 mglkglday

Group 2 Females: 246 mg/kg/day
Group 3 Females: 991 mglkglday
Group 4 Females: 1713 mg/kg/day
Group 5 Females: 2460 mg/kg/day

Toxic Response/Dose Level:

Mortality: One rat was killed for humane reasons on Day 5 of Week 4, following a series of convulsive-like episodes. This mortality was considered to be related to treatment. Macroscopic

examination revealed stained/moist fur, enlarged cervical lymph nodes, an enlarged left testis, minimal adipose tissue and 2 white nodules near to the limiting ridge of the gastric mucosa. There were, however, no clear indications of the origin of the convulsive episodes and the brain of this animal appeared normal under microscopic examination.

Clinical Signs: Clinical signs considered to be related to treatment included yellow or brown stained fur, a hunched posture, body tremors and convulsive-like episodes. In general, the highest incidence of clinical signs occurred amongst rats assigned to Group 5 late in the pre-dose period.

Bodvweight: An adverse effect on group mean bodyweight was noted for both sexes receiving 1750 or 2500 mg/kg/day from Week 1 and in males receiving 250 or 1000 mg/kg/day during Weeks 6 to 13. Females receiving 1000 mg/kg/day showed an adverse effect on bodyweight gain during Week 1 only. A general dosage-relationship was apparent in the affected groups. Small reductions in food intake in Weeks 1 and 2 were insufficient to fully account for the adverse effect on bodyweight. Overall to Week 13, both sexes receiving 1750 or 2500 mg/kg/day showed a statistically significantly lower weight gain in comparison with Controls (with a dosage-relationship). The marginally lower weight gain for males treated with 250 mg/kg/day and females receiving 1000 mg/kg/day was considered insufficient to be of toxicological importance. Moreover, the final bodyweights for males and females fed 1000 mg/kg/day were only 3% and 4% decreased, respectively.

Food Consumption: There appeared to be an adverse palatability reaction over the first few days of treatment, with rats in all treated groups spilling a significant quantity of diet from the food hoppers. The amount of spillage appeared to be approximately dosage-related. Towards the end of the first week, the amount of spillage decreased. Group mean food consumption by males receiving 1750 or 2500 mg/kg/day was notably reduced in Week 1 and slightly reduced in Week 2 (with a dosage-relationship). Females receiving 2500 mg/kg/day showed a marginally lower consumption in Week 1 only. In subsequent weeks food consumption by treated groups was considered to be normal and overall consumption to Week 13 and throughout the Recovery period was essentially comparable to that of the Controls.

Water Consumption: There was no effect of treatment.

Ophthalmoscopy: There were no changes at Week 13 considered to be related to treatment. All findings were characteristic of the age and strain of animals employed. Ophthamoscopy was therefore not performed during the recovery period.

Hematoloay: The minor intergroup differences in mean erythrocyte parameters, some of which achieved statistical significance, were considered of no toxicological importance in the absence of a dosage-relationship and/or consistency between the sexes and as the majority of the results were characteristic for rats of this age. The only notable individual finding was an apparent anaemia in two rats dosed at 2500 mg/kg/day, characterized by low PCV, haemoglobin, erythrocyte count and mean corpuscular haemoglobin concentrations together with a high reticulocyte count, indicative of a regenerative anaemia. By Week 4 of the Recovery period, the regenerative anaemia had fully recovered. The anaemia is considered related to treatment and indicative of inadequate compensatory erythropoiesis in a small number of susceptible rats. All individual white cell data and the results of clotting tests were normal for rats of this age and strain, therefore the minor intergoup differences are considered to be coincidental and of no toxicological importance. A single animal showed a very low platelet count at Week 13 which slightly influenced the group mean result. In isolation, this single low result cannot be attributed to treatment, There were no other notable findings at Week 13.

Biochemistry: Plasma glucose levels at Week 13 were towards or below the lower end of the normal range in a number of females at all dosage levels and in males receiving 1750 or 2500 mg/kg/day, leading to a degree of statistical significance for males receiving 1750 mg/kg/day and

both sexes treated with 2500 mg/kg/day. Intergroup differences were, however, small and only in 3 animals dosed at 2500 mg/kg/day showed remarkably low individual results. In Recovery Week 4 several individuals previously receiving 2500 mg/kg/day continued to exhibit low plasma glucose levels and although the group mean result was below that of the Controls to a statistically significant degree, the actual group mean result had improved slightly from that recorded at Week 13, indicating a general trend for recovery. A number of males receiving 2500 mg/kg/day showed very low total plasma protein levels at Week 13, principally as a result of lower B-globulin levels. By Recovery Week 4 all group mean and individual plasma protein data were considered to be characteristic for the age and strain of rat employed. Plasma urea nitrogen was very low for 2 females receiving 1750 mg/kg/day and 5 females receiving 2500 mg/kg/day in Week 13 and group mean values achieved a level of statistical significance. By Recovery Week 4 all individual data were considered unremarkable and the slightly lower group mean result for females previously treated with 2500 mg/kg/day was considered to be coincidental. A number of males receiving 2500 mg/kg/day showed notably high AP values at Week 13 and the group mean result was slightly elevated, but did not achieve a level of statistical significance. Group mean GPT and GOT activities were increased to a statistically significant degree for males receiving 2500 mg/kg/day in Week 13. GPT, GOT and OCT activities of males previously receiving 2500 mg/kg/day had returned to normal levels by Recovery Week 4. Females receiving 2500 mg/kg/day also showed slightly raised group mean GPT and GOT activities at Week 13, principally as a result of high activities in a single individual; however, as one Control female also showed a similar result these elevated activities cannot be attributed to treatment in females.

Gross pathology: Staining of the fur in the genital region was noted at the Main kill in 3110 males and 5/10 females receiving 2500 mg/kg/day. This is considered to be related to the findings detected clinically, but is of little toxicological importance. There were no notable macroscopic findings in animals killed upon completion of the 4-week recovery period.

Organ Weight Changes: Liver weight as a percentage of terminal bodyweight was notably increased for a number of animals of both sexes receiving 2500 mg/kg/day at the Main kill. A corresponding increase in group mean values adjusted for, and as a percentage of, terminal bodyweight was noted for both sexes receiving 2500 mg/kg/day and generally achieved a level of statistical significance. Upon completion of the Recovery period, the intergroup differences in liver weight were small and considered to indicative of complete recovery. The increased liver weight at the highest doses correlated with the observation of minimal liver cell hypertrophy. In the absence of corroborative pathological findings, the intergroup differences in absolute organ weight for all other parameters at the Main and Recovery kills were considered not to be of toxicological importance and individual values were generally characteristic for rats of the age employed. The improved bodyweight performance of rats previously receiving 2500 mg/kg/day during the Recovery period lessened the impact of bodyweight upon relative organ weight parameters at the Recovery kill.

Histopathology: Treatment Period - *Liver:* Periportal hepatocyte hypertrophy was seen in the majority of males and an occasional female rat receiving 2500 mg/kg/day. This finding is not inconsistent with the minimal increase in individual and group mean liver weights for males and females receiving 2500 mglkglday and the increased values recorded for liver transaminases for male rats receiving 2500 mglkglday. Spleen: Haemosiderosis is a normal physiological response to red cell turnover in rats and is most frequently present to a minimal degree in control rats. However, an increased incidence and degree of haemosiderosis was seen in male and female rats receiving 2500 and 1750 mg/kg/day. This was considered to be a treatment-related exacerbation of this physiological change. *Caecum:* Minimal epithelial hyperplasia was seen in a proportion of male and female rats receiving 2500 mglkglday. Colon: Minimal epithelial hyperplasia was seen in an occasional male rat receiving 2500 mglkglday. Stomach: Focal epithelial hyperplasia or hyperkeratosis of the non-glandular region adjacent to the limiting ridge was seen in some male rats.
Recovery Period - *Spleen:* The degree of haemosiderosis was still increased in male and female rats receiving 2500 mg/kg/day, although there was evidence of recovery with slight being the

highest grade recorded. *Liver*, stomach, caecum **and** colon: No treatment-related changes were detected in rats receiving 2500 mg/kg/day after the 4-week recovery period. The periportal hepatocyte hypertrophy seen in the liver, the lesion in the non-glandular stomach and the minimal epithelial hyperplasia seen in the caecum and colon of rats in this treatment group at the end of the treatment period were therefore considered to be reversible.

Statistical Analysis: All statistical analyses were carried out separately for males and females. Data relating to food and water consumption were analyzed on a cage basis. For all other parameters, the analyses were carried out using the individual animal as the basic experimental unit. Food consumption data were analyzed using cumulative totals and water consumption data were analyzed as the total recorded intake over a selected time period, expressed on a weekly basis. Bodyweight data were analyzed using weight gains.

Interpretation/Conclusion: Benzoflex 2-45 was administered to rats by dietary admixture to achieve dosages of 0, 250, 1000, 1750 or 2500 mg/kg/day over 13 weeks. Selected Control and Group 5 animals were subsequently maintained off dose for 4 weeks to assess reversibility of any treatment related changes.
There were no findings of toxicological importance at a dosage of 1000 mg/kg/day or below.
In animals receiving 1750 or 2500 mglkglday, there was an adverse effect on bodyweight gain, changes in clinical pathology parameters and an increased incidence/degree of haemosiderosis in the spleen. In addition, at 2500 mglkglday, a few treatment-related clinical signs were evident, minimal periportal hepatocyte hypertrophy was noted in both sexes. Plasma enzyme activities (transaminases and/or AP or OCT) were elevated at Week 13 in rats receiving 1750 or 2500 mglkglday with an associated minimal increase in liver weight, At necropsy, minimal periportal hepatocyte hypertrophy was detected only at 2500 mg/kg/day. The slight effects in the liver may be a physiological adaptation to treatment at the highest doses. Following 4-weeks recovery, most enzyme activities were normal, liver weights were unremarkable and there was no residual hepatic pathology.
Epithelial hyperplasia was detected in the colon of males and the caecum of both sexes. In general, however, dosages of up to 2500 mg/kg/day of Benzoflex 2-45 were tolerated.
When selected animals previously receiving 2500 mg/kg/day were maintained off-dose for 4-weeks, all treatment related changes showed evidence of, or complete, recovery.

Data Quality: 1, Reliable without restrictions

References: Benzoflex 2-45. Toxicity to rats by Dietary Administration for 13 weeks with Subsequent 4-Weeek Recovery Period for Selected Animals. Huntingdon Life Sciences. 1999.

DEVELOPMENTAL TOXICITY

Test Substance:

Diethylene Glycol Dibenzoate	97.67%
Dipropylene Glycol Dibenzoate	1.34%
Ethylene Glycol Dibenzoate	0.15%
Propylene Glycol Dibenzoate	0.11%
n-Propyl Benzoate	0.054%
Diethylene Glycol Monobenzoate	0.047%
Unknown #1	0.19%

Method: US EPA 870.3700 Harmonized Guideline, with the following exception: the guideline states that "Evaluation of the dams during cesarean section and subsequent fetal analyses should be conducted without knowledge of treatment group in order to minimize bias". Evaluation was made with knowledge of the treatment group, as procedures are already in place to minimize bias during these portions of the study. These procedures include routine reviews of

necropsy technicians evaluation skills and scientific peer review of at least 25% of the raw data of the fetal analyses, including examination of serial sections for visceral anomalies and examination of fetal skeletons.

GLP: **Yes**

Year Performed: *1998*

Laboratory: Huntingdon Life Sciences

Species/Strain: Rat, Sprague-Dawley

Route of Administration: Oral (gavage)

Dosages: 0,250, 500 and 1000 mg/kg/day

Number and Sex: 22 Females / group

Exposure period: Days 6 - 19 of gestation inclusively

Frequency of Treatment: Daily

Control Group: Corn Oil Vehicle

Duration of Test: Necropsy at Gestation Day 20

Statistical Evaluation: Statistical tests, employing analysis of variance followed by an inter-group comparison with the Control, were performed on the following parameters: Bodyweight change, bodyweight change adjusted for gravid uterine weight, food consumption, litter data, litter weight, fetal weight and placental weight.

Dependant on the heterogeneity of variance between treatment groups, parametric tests (analysis of variance Snedecor and Cochran 1967) followed by Williams' test (Williams 1971/2) or nonparametric tests (Kruskal-Wallis, Hollander and Wolfe 1973) followed by Shirley's test (Shirley 1977) were used to analyze these data, as appropriate.

For litter data (excluding fetal, litter and placental weights) and implantation loss, due to the preponderance of non-normal distributions, non-parametric tests are generally the most consistent and were routinely used.

All significant (i.e.$p<0.5$) inter-group differences from the Control are reported only where supported by a significant analysis of variance (i.e. $p<0.0.5$)

HOLLANDER, M and WOLFE, D.A. (1973) *Non-parametric statistical methods.* Publ. J. Wiley and Sons, New York. KRUSKAL-WALLIS and JONCKHEERE tests: 114-132.

SHIRLEY, E. (1977) A non-parametric equivalent of William's test for contrasting increasing dose levels of a treatment. *Biometrics, 33: 386-389.*

SNEDECOR, G.W. and COCHRAN, W.G. (1967) *Statistical methods.* 6th ed. The Iowa State University Press.

WILLIAMS, D.A. (197112) William's test for comparing the effect of increasing doses of substance with a zero dose. *Biometrics 27: 103-I 17. Biometrics, 28:* 519-531.

REMARKS

Age at Study Initiation: 10 to 11 weeks of age

Test Substance Preparation: Benzoflex 2-45 was formulated in corn oil. Formulations were prepared in batches of up to one week and stored refrigerated at approximately 4°C prior to use.

Clinical Observation (Maternal): All animals were observed at least twice daily throughout study for any visible signs of reaction to treatment. Observations associated with dosing were also recorded during the treatment period according to the following schedule: 1) pre-dosing, 2) On return of animal to home cage, 3) After dosing each group, 4) 1 to 2 hours after completion of dosing all groups, 5) as late as possible in the working day. Maternal bodyweight was measured on Days 0,3, 6 to 17 inclusive and 20 after mating. Food consumption was recorded for the periods Days O-2, 3-5, 6-8, 9-l 1, 12-14, 15-16 and 17-19 after mating.

Mating Procedure: Females were paired on a one-to-one basis with stock males of the same strain. Each morning following pairing, the trays beneath the cages were checked for ejected copulation plugs and a vaginal smear was prepared from each female and examined for presence of spermatozoa. The day on which a sperm positive vaginal smear or at least 3 copulation plugs were found was designated Day 0 of gestation.

Terminal Observations (Maternal): On Day 20 after mating, the females were killed by inhaled carbon dioxide for examination of their uterine contents. Each animal was first examined macroscopically for evidence of disease or adverse reaction to treatment and specimens of abnormal tissue were retained. The reproductive tract, complete with ovaries, was dissected out and the following recorded: 1) Gravid uterine weight – uterus with cervix, 2) number of corpora lutea in each ovary (assessed prior to removal), 3) number of implantation sites, 4) number of resorption sites (classified as early or late), 5) number and distribution of fetuses in the uterine horn.

Fetal Examination: Each fetus was weighted, sexed and examined for any external abnormalities. Individual placental weights and placental abnormalities were recorded. Fetuses were killed by chilling on a cool plate. The neck and thoracic and abdominal cavities of approximately half of each litter were dissected and examined. Fetal changes were recorded and the offspring eviscerated prior to fixation in Industrial Methylated Spirit. After fixation, fetuses were processed, stained with Alizarin Red and skeletal development assessed. The remaining fetuses in each litter were placed in Bouin's fixative, subjected to free hand serial sectioning and examination for visceral changes.

RESULTS

Maternal Toxicity

NOEL: 1000 mg/kg/day

Clinical Signs: The general condition of females at all dosages remained satisfactory throughout the study and there were no deaths. Salivation after dosing was observed at all dosages. The incidence was dosage related but this finding was not considered to be of toxicological importance. At 1000 mg/kg/day, there were no detectable signs of maternal toxicity; there were no maternal deaths and all females had a live litter at sacrifice.

Litter Responses and Fetal Changes

Prenatal development NOAEL: 500 mg/kg/day. Although a small number of fetuses with cervical ribs at 1000 mg/kg/day precludes defining this dosage as a NOEL for developmental anomalies, there were no findings at this dosage that were considered indicative of any substantial disturbance of morphological development.

Fetal Growth and Development NOEL: 250 mg/kg/day

Post-implantation loss was higher in all treated groups compared to the concurrent Control, differences attaining significance at 500 and 1000 mg/kg/day. However values were comparable with recent background control data and it is considered that the test groups were disadvantaged by a particularly high survival rate in the Control. It was concluded that in *utero* survival had not been adversely affected by treatment since live litter size was unaffected and was similar in all groups.

At 1000 mg/kg/day mean fetal weights, and consequently litter weight were slightly lower than the Control, combined with fetal weight and female fetal weight attaining statistical significance. Placental weight was comparable with the Control.
At 1000 mg/kg/day 4 fetuses showed cervical ribs, this incidence being higher than the concurrent Control and marginally outside the current background control data. Although the incidence of this finding was relatively low, it is considered that a treatment relationship could not be ruled out. There was a clearer increase in the incidence of incomplete ossification, principally affecting the cranial centers, sacrocaudal vertebral arches, $5^{th}/6^{th}$ sternebral centers and pelvic bones compared with the concurrent Control.

At 500 mg/kg/day the incidence and distribution of skeletal anomalies did not indicate any obvious adverse effect of treatment. There was a slight increase in the incidence of incomplete ossification of the $5^{th}/6^{th}$ sternebrae centers, however the incidence was within that seen for the background data and any relationship to treatment was considered equivocal.

Analytical Results:

Group:	1	**2**	**3**	**4**
Dosage: (mg/kg/day)	0	**250**	**500**	**1000**
Adult Females				
Females with sperm	22	22	22	22
Pregnant Females	22	22	22	22
Evaluated Pregnant Females	**22**	**22**	**22**	**22**
Litters – group mean values				
Corpora lutea	15.8	16.5	16.1	16.1
Implantation	14.9	15.4	15.5	15.0
Resorptions	0.4	0.8	1.0a	1.0a
Live fetuses	14.5	14.6	14.5	14.0
Sex ratios of fetuses (%)				
Male	44.8	45.7	49.5	47.9
Weight of fetuses (g)				
Male	3.85	3.88	3.85	3.72
Female	3.63	3.68	3.63	3.50a
Overall	3.73	3.76	3.74	3.60a
Important Fetal Findings	At 1000 mg/kg/day treatment was associated with a reduction in fetal weight and accompanying increased incidence of incomplete ossification, principally affecting the cranial centers, sacrocaudal vertebral arches, $5^{th}/6^{th}$ sternebral centers and pelvic bones compared with the concurrent Control. There was also a slight increase in the number of fetuses with cervical ribs, the incidence marginally outside current background Control data.			

CONCLUSIONS

Fetal weight at 1000 mg/kg/day was marginally lower than the Control; differences occasionally attained statistical significance although weights were generally comparable with background control data. In addition, there was also an increased incidence of incomplete ossification, principally affecting the cranial centers, sacrocaudal vertebral arches, $5^{th}/6^{th}$ sternebral centers and pelvic bones compared with the concurrent Control. Collectively, these findings are suggestive of a retardation of fetal growth at this dosage.

Although fetal weight was unaffected by treatment at 500 mglkglday, there was a greater incidence of fetuses with incomplete ossification of the 5^{th} and/or 6^{th} sternebrae at this dosage, compared to the concurrent Control. In light of the findings observed at 1000 mg/kg/day, an effect of treatment can not be discounted, as this incidence was comparable with that seen in the current background control data, it is considered equivocal. These findings are consistent with a slight delay in development rather than representing permanent structural changes and, at the incidence seen in this study, are generally not considered to be of toxicological significance.

The incidence of cervical ribs at 1000 mg/kg/day in association with treatment is debatable; there was no accompanying change in vertebral configuration and only a very small number of fetuses at the limit dosage of 1 000mg/kg/day were affected.

In conclusion, it is considered that 1000 mg/kg/day is the no-effect-level for maternal toxicity.

While the occurrence of a small number of fetuses with cervical ribs at 1000 mg/kg/day precludes defining this dosage as a no-observed effect-level for developmental anomalies, in all other respects the no-adverse-effect-level for pre-natal development is concluded to be 1000 mg/kg/day.

The no-adverse-effect-level for all aspects of pre-natal development is concluded to be 500 mg/kg/day.

The no-observed-effect level for fetal growth and development was 250 mg/kg/day.

RELIABILITY: 1, Reliable without restrictions

REFERENCES: Benzoflex 2-45. Study of Prenatal Development in the CD Rat by Oral Gavage Administration. Huntingdon Life Sciences. 2000.

REPRODUCTIVE TOXICITY

Test Substance:	Diethylene Glycol Dibenzoate	97.67%
	Dipropylene Glycol Dibenzoate	1.34%
	Ethylene Glycol Dibenzoate	0.15%
	Propylene Glycol Dibenzoate	0.11%
	n-Propyl Benzoate	0.054%
	Diethylene Glycol Monobenzoate	0.047%
	Unknown #1	0.19%
Method:	OECD 416 (1983), USEPA OPPTS 870.3800 (1998)	
Type:	Two-generation	
GLP:	**Yes**	
Year Performed:	1999 and 2000	

Laboratory:	Huntingdon Life Sciences
Species/Strain:	Rat, Sprague-Dawley (CD-IGS)
Route of Administration:	Dietary – Continuous
Doses:	0, 1000, 3300 or 10000 ppm throughout two generations
Sex:	Males and females
Control Group:	Yes, basal diet without the test material
Frequency:	Continuously in diet
Duration:	Approximately 38 weeks

Premating Exposure (males and females):
FO Generation: 10 weeks before pairing and throughout mating, gestation, littering and lactation
F1 Generation: 11 weeks before pairing and throughout mating, gestation, littering and lactation

Statistical Methods:
Where considered appropriate, significance tests employing analysis of variance followed by an intergroup comparison with the control were performed. These were performed on the following parameters: bodyweights and bodyweight change, food consumption of females during gestation and lactation, litter data including offspring weights, sexual development data, and organ weights.

For data recorded and/or processed by the Xybion computer system (adult organ weights and weekly bodyweight change) for the parental animals, homogeneity of variance was assessed using Barlett's test. Whenever this was found to be statistically significant a Beherens-Fisher test was used to perform pairwise comparison, otherwise a Dunnett's test was used.

For bodyweight, food consumption data during gestation and lactation, litter data, sexual development data, seminology data and offspring organ weights, statistical analysis was performed using the Startox program developed by Huntingdon life Sciences. Dependant on the heterogeneity of variance between treatment groups, parametric tests (analysis of variance, Snedecor and Cochran 1967) followed by Williams' test (Williams' 1971/2) or non-parametric tests (Kruskal- Wallis, Hollander and Wolfe 1973) followed by Shirley's test (Shirley 1977) were used, as appropriate.

Where 75% or more of the values for a given variable were the same, a Fisher's exact test (Fisher 1950) was used.

Significant (i.e. $p<0.05$) inter-group differences from the Control are reported where supported by a significant analysis of variance (i.e. $p<0.05$).

FISHER, R.A. (1950) Fisher's exact test 2x2 contingency table: *Statistical* Methods for *Research Workers,* para. 21.02 Oliver and Boyd, Edinburgh.

HOLLANDER, M and WOLFE, D.A. (1973) *Non-parametric* statistical *methods.* Publ. J. Wiley and Sons, New York. KRUSKAL-WALLIS and JONCKHEERE tests: pages 114-*132.*

SHIRLEY, E. (1977) A non-parametric equivalent of William's test for contrasting increasing dose levels of a treatment. *Biometrics, 33: 386-389.*

SNEDECOR, G.W. and COCHRAN, W.G. (1967) *Statistical* methods. 6'" ed. The Iowa State University Press.

WILLIAMS, D.A. (1971/2) William's test for comparing the effect of increasing doses of substance with a zero dose. *Biometrics 27: 103-I 17. Biometrics, 28: 519-531.*

REMARKS

Groups of rats were administered the test compound continuously in their diet at levels of 1000, 3300 or 10000 ppm throughout the two generations. A fourth group received the basal diet without the test material and served as the Control.

The FO generation, which comprised 32 males and 32 females in each group, received the treated diet for 10 weeks before pairing and throughout mating, gestation, littering and lactation. Offspring survival, growth and sexual maturation were evaluated. From the litters 28 male and 28 female offspring per group were selected to form the F1 generation. Both sexes received similarly treated diets as their parents for a minimum of 10 weeks from selection, throughout pairing, gestation, littering and lactation. F2 offspring were monitored for survival and development until weaning.

All FO and F1 adult animals were subjected to a detailed necropsy, the reproductive organs and selected organs were weighed and retained. Sperm motility and morphology was assessed from samples obtained from the left vas deferens and sperm counts were determined for the left epididymis and testis for all FO and F1 males. Histopathological examinations were performed on designated tissues from 10 parent males and 10 parent females in the Control and high dose groups, and abnormal tissues from all other parental animals.

Unselected F1 offspring were killed on or after 31 days of age and F2 offspring were killed on Day 21 of age. These offspring were subjected to necropsy examination and histopathological examinations were performed on the macroscopic abnormalities observed. Where possible, one male and one female from each litter were subjected to necropsy examination, the reproductive organs retained, and the brain, spleen and thymus weighed and retained.

Mating Procedure:
Following the scheduled period of treatment (10 weeks of treatment for the FO generation; 11 weeks after selection for the F1 generation), males and females from within the same treatment groups were paired on a one-to-one basis for a period up to 3 weeks. If there were no positive indication of mating after 14 days and the females had shown evidence of estrous at the time, the male partner was replaced by a proven male from within the same group. Care was taken to avoid pairing siblings. Each morning following pairing, the trays beneath the cages were checked for ejected copulation plugs and a vaginal smear was prepared from each female and examined for the presence of spermatozoa and the stage of the estrous cycle. The day on which evidence of mating was found was designated Day 0 of gestation.

Once mating occurred, the males and females were generally separated and smearing was discontinued. However after inconclusive mating, smearing continued up to 5 days to confirm positive mating.

Parameters assessed during FO and F1:
All animals were observed at least twice daily throughout the study and any visible signs of reaction to treatment were recorded. A more detailed weekly examination was performed throughout the treatment period. All animals found dead or killed for reasons of animal welfare were subjected to a thorough macroscopic examination of the visceral organs and specimens of abnormal tissues were retained. Males were weighed on the day that treatment commenced (FO) or the formal start of the generation (F1), then weekly thereafter. FO and F1 females were weighed on the same schedule until mating was detected and then on Days 0, 6, 13 and 20 after

mating and on Days 1, 4, 7, 14 and 21 of lactation. Food consumption was recorded on a cage basis for FO and F1 males and females weekly before pairing. Food consumption for females after mating was recorded daily on an individual basis on Days O-5, 6-12 and 13-19 after mating. Food consumption for FO females should have been recorded on Days 1-3, 4-6, 7-l 3, and 14-20 of lactation but was recorded on Days 1-6, 7-13, 14-17 and 18-20 in error. Food consumption for F1 females was correctly recorded on Days 1-3, 4-6, 7-13, and 14-20 of lactation. After Day 14 of lactation, food intake is increasingly influenced by the offspring and is no longer an accurate reflection of maternal intake.

Estrous cycles FO and F1:

For 22 days before pairing of FO and 29 days before pairing the F1 generations, daily vaginal smears were taken from all females and examined to establish the duration and regularity of the estrous cycle. After pairing with the male, smearing was continued until evidence of mating was observed. Following weaning, daily vaginal smears were taken from all females on Days 22 to 28 after birth prior to necropsy and use to determine the stage of the estrous cycle at termination. Females whose litters died before weaning were retained and vaginal smears taken on their theoretical Days 22-28 and killed on Day 28, as for those with surviving litters. Any females that failed to mate, mated but were not pregnant or failed to litter were retained and vaginal smears taken for 7 days, starting on the day on which the first batch of females with live litters for that generation had vaginal smears taken. These animals were killed with the first batch of females with a litter for that generation.

Seminology FO and Fl:

After sacrifice, sperm motility, sperm morphology, sperm count and homogenization-resistant spermatids were recorded. Specifically, the % of motile and progressively motile sperm reported; % of normal sperm and abnormal sperm reported; the concentration and total number of sperm reported: and the concentration and total number of spermatids reported.

Parameters Assessed during F1 and F2:

All offspring were examined at approximately 24 hours after birth (Day 1 of age) and the following were recorded for each litter: number of offspring (live and dead), individual bodyweights of live offspring, sex ratio and observations of individual offspring. Litters were observed daily for evidence of abnormal appearance or behavior. Daily records were maintained for mortality and consequent changes in litter size. Whenever possible, any offspring found dead were examined externally and internally. Litters containing more than 10 offspring were culled by random selection to 10 (where possible 5 males and 5 females) on Day 4 of age. Individual F1 and F2 offspring were weighed on Days 1,4,7,14 and 21 of age. Selected female F1 generation offspring were examined daily from Day 28 of age until vaginal opening occurred. Bodyweights were recorded on the day of vaginal opening for each animal. Selected male F1 generations offspring were examined daily from Day 35 of age until balano-preputial separation occurred. Bodyweight was recorded on day of start and completion of separation for each animal.

All parental animals were subject to a detailed macroscopic examination for evidence of disease or adverse reaction to treatment. The necropsy procedure included a review of the history of each animal, and a detailed examination of the cranial, thoracic, abdominal and pelvic cavities and their viscera. The external and cut surfaces of the organs and tissues were examined, either before or after weighing as appropriate. The number of uterine implantation sites was recorded for the adult females. Abnormalities interactions and changes were noted, the requisite organs weighed and the required tissue samples preserved in fixative. Unselected F1 offspring and F2 offspring were examined macroscopically for evidence of disease or adverse reaction to treatment and appropriate organs weighed and retained. Any abnormal tissues were also retained.

The following tissues were microscopically examined for 10 parent males and 10 parent females of Groups 1 and 4 sacrificed on completion of the schedule treatment period and for all adult animals killed or dying before schedules termination: adrenal glands, epididymis (right),

mammary glands (caudal), ovaries with oviduct (left and right), pituitary, prostrate (ventral lobe), seminal vesicles and coagulated gland, testis (right), uterus with cervix, vagina. Mammary glands were retained from females with total litter loss.

RESULTS

The general condition of the FO and F1 adults was satisfactory throughout. There were occasional deaths in adults animals in both generations but none that were considered to be related to treatment. No adverse effects of treatment were observed on bodyweight gain, food intake, or the efficiency of food utilization for males in either generation at any of the inclusion levels investigated. No adverse effects of treatment were observed on bodyweight gain, food intake, or the efficiency of food utilization during the pre-pairing treatment period for females in either generation at any of the inclusion levels investigated.

Overall bodyweight gain and food intake during gestation and lactation were not noticeably affected by treatment in either generation. At 10000 ppm, FO and F1 females showed lower bodyweight gain, compared to Control, during the first 4 days after their litters were born; subsequent weight gain however, was essentially similar or superior and values were comparable to Control by the end of lactation. A similar observation of lesser magnitude was also seen in F1 dams at 3300 ppm.

There was no adverse effect of treatment on estrous cycles, mating performance or fertility in either generation at any of the inclusion levels investigated. In both generations, gestation length and the parturition process appeared unaffected by treatment. The return of females to estrous cycling following lactation was not influenced by treatment in either generation.

Sexual maturation, as assessed by the age and bodyweight at the time of attainment of vaginal opening and balano-preputial separation, was not affected by treatment of Benzoflex 2-45.

There was no overt effect on corpora lutea count, implantation numbers, litter size at Day 1 and subsequent survival to weaning in either generation. In the F1-F2 generation there was a high number of litters which failed to rear their young to weaning but this was considered to be consistent with the pattern of total litter losses observed to be inherent in the CD UK strain and unrelated to treatment.

Mean F1 offspring bodyweight at Day 1 and subsequent mean weight gain to weaning were not clearly affected by treatment. At 10000 ppm however, mean pup weight at Day 1 was marginally lower than the Control, despite a slightly lower litter size, and there was also a suggestion of lower weight gains between Days 14-21 of age; this period representing the transition to direct exposure to the test material as the offspring begin to consume the parental diet. For F2 offspring bodyweight on Day 1 was again lower and subsequent bodyweight gains to weaning were also lower than the control; it is considered that this may be related to treatment. At lower dosages bodyweight of the F2 offspring on Day 1 and subsequent gains to weaning were not adversely influenced by treatment.

Macroscopic necropsy examination and microscopic examination of any abnormalities observed for the unselected F1 offspring or the F2 offspring did not indicate any adverse effect of treatment. At 10000 ppm mean absolute and bodyweight relative values of the spleen in male and female F2 offspring was lower than the Control. Mean spleen weights in the F1 offspring were comparable to Control, as were brain and thymus weights for both F1 and F2 offspring.

Organ weights, macropathology and subsequent histopathological assessment for the FO and F1 adults showed no adverse effects of treatment. Sperm analysis in males of both generations was also not adversely influenced by treatment.

Analytical Results:

Group:	1	2	3	4
Dietary Concentration (ppm):	**0**	**1000**	3300	10000
FO Parental Animals				
Number females with normal estrous cycle	31	29	31	30
Number males/females paired 1 :1	32	32	32	32
Number females with sperm	32	32	32	32
Pregnant females	32	31	32	30
Females with delivery	32	30	32	29
Decedents (females)	1	1	1	1
Important parental findings				
Low bodyweight gain Days l-4 of lactation	17	13	10	3
F1 Parental Animals				
Number females with normal estrous cycles	24	27	28	26
Number male/female paired 1 :1	28	28	28	28
Number females with sperm	28	28	28	28
Pregnant females	27	25	28	27
Females with delivery	27	24	28	27
Decedents (female)	0	1	2	0
Important parental findings				
Low bodyweight gain Days l-4 of lactation	8	6	3	-3
FO - F1 Litters				
Implantations, assessed at termination - mean	15.1	15.4	15.2	14.1
Live litters	31	30	31	28
Sex ratio Day 1 after birth (as %M) - mean	55.5	51.4	47.9	52.1
Number offspring Day 4 after birth (before culling) - mean	12.7	13.3	12.6	12.2
Number offspring Day 4 after birth (after culling) - mean	**9.7**	**9.9**	**9.7**	**9.8**
Surviving litters at Day 4 after birth	31	28	**29**	27
Number offspring at Day 21 - mean	9.5	9.3	9.4	9.6
Surviving litters at Day 21 after birth	31	28	29	27
Weight at birth (g) - mean				
Males	6.5	6.0	6.1	6.2
Females	6.2	5.6	5.7	5.9
Weight at weaning (g) - mean				
Males	48.8	48.6	46.9	46.5
Females	46.6	46.9	45.1	44.3
Important findings for F1 post-weaning progeny -- to sexual maturation				
None considered to be related to treatment				
F1 - F2 Litters				
Implantations, assessed at termination - mean	13.4	14.1	13.3	13.5
Live litters	27	22	25	26
Sex ratio Day 1 after birth (as % M) mean	51.8	47.7	44.3	50.4
Surviving litters at Day 4 after birth	23	20	18	19
Yumber offspring Day 4 after birth (before culling) - mean	10.2	11.3	10.5	11.5
Number offspring Day 4 after birth (after culling) - mean	**9.0**	**8.9**	8.8	9.4
Surviving litters at Day 21 -weaning	22	20	16	17
Number offspring at Day 21 - mean	8.7	8.7	8.4	9.4
Weight at birth (g) - mean				
Males	6.4	6.0	6.3	5.9
Females	6.0	5.7	5.8	5.6
Weight at weaning (g) - mean				
Males	49.7	46.5	45.7	43.5

Females	46.8	44.4	42.2	41.6
Weight gain Day 1-21 - mean				
Males	43.3	40.6	39.4	37.6
Females	40.8	38.8	36.4	36.1
Important findings for F2 progenv				
Bodyweight relative spleen weights (% bodyweight) on Day 21 of age				
Males	0.4512	0.4230	0.4454	0.4110
Females	0.4785	0.4848	0.4864	0.3975

Conclusion

Dietary administration of the test material at concentrations of 1000, 3300 or 10000 ppm was generally well tolerated by the FO and subsequent F1 parental animals and their respective progeny. Exposure to the test material was in line with expectation throughout both generations; fluctuations reflected the different physiological status of the animals and were, predictably, highest for females during peak lactation and in young animals. The three-fold interval between treatment levels was maintained throughout. There were no obvious toxicological effects of treatment for the two generations on the general condition of the parental animals although a slight disturbance in the pattern of maternal weight change was noted at 10000 ppm in both generations and at 3300 ppm in the F1 generation. There was no effect on fertility and reproductive performance at any of the dietary inclusion levels in either generation.

Litter parameters at birth of the F1 and F2 progeny and their survival to weaning showed no apparent detrimental effects of treatment. However, for the F2 offspring at 10,000 ppm there was a reduction in weight gain from birth to weaning.

No abnormal findings were apparent at necropsy of the FO or F1 parental animals, the post-weaned unselected F1 offspring or the F2 offspring. Organ weight assessment of the FO and F1 parent animals did not suggest any adverse effects on any organs. Assessment of spermatogenesis and histopathology in both parental generations showed that there were no injurious effects on these testes or other reproductive organs. Furthermore, detailed histopathological examination of the tissues from both sexes in both generations did not reveal any adverse effects of treatment. The only possible effect of treatment detected at assessment of organ weights from F1 and F2 offspring was lower absolute and bodyweight relative spleen weights among F2 males and females compared with Controls.

The evidence from this study suggested that a dietary concentration of 10,000 ppm should be considered as the No-Observed-Adverse-Effect-Level (NOAEL) for the FO and F1 parent animals. The No-Observed-Adverse-Effect-Level (NOAEL) for the developing offspring is considered to be 3300 ppm. The No-Observed-Effect-Level (NOEL) for reproductive parameters is considered to be 10000 ppm.

RELIABILITY: 1, Reliable without restrictions

REFERENCES: Benzoflex 2-45. Study of Reproductive Performance in CD Rats Treated Continuously through Two Successive Generations by Dietary Administration. Huntingdon Life Sciences. 2001.

AMES MUTAGENICITY

Test Substance: See boiling point for purity

Solvent: DMSO

Study Type: Bacterial reverse mutation assay

Test Method: OECD 471,472

GLP: **Yes**

Year Performed: 1997

Laboratory: Huntingdon Life Sciences

Species/Strain:

S. typhimurium:	TA 1535 his G46 rfa uvrB
	TA 1537 his C3076 rfa uvrB
	TA 98 his D3052 rfa uvrB pKM101
	TA 100 his G46 rfa uvrB pKM101
E. coli	CM891 WP2 trp uvrA pKM101

Concentrations: 5000, 1500, 500, 150, 50, 15, 5 μg/plate

Metabolic Activation: Sprague-Dawley rat liver

Quantity of Activator: 0.5 ml

Induction: Stimulated by Aroclor 1254

Criteria for Evaluating Results:

(a) If treatment with test substance produces an increase in reverent colony numbers of at least 2 times the concurrent solvent controls with some evidence of a positive dose relationship, in a specific bacterial strain reproduced with or without S9 mix, it is considered to show evidence of mutagenic activity in this test system. No statistical analysis is performed.

(b) If treatment with a test substance does not produce reproducible increases of at least 1.5 times the current solvent controls, at any dose level, with any bacterial strain, it is considered to show evidence of mutagenic activity in this test system. No statistical analysis is performed.

(c) If the results obtained fail to satisfy the criteria for a clear "positive" or "negative" response given in paragraphs (a) and (b), additional testing may be performed in order to resolve the issue of the substance's mutagenic activity in this test system. Modifications to the experimental method will usually be considered, such as the use of a narrower dose range and different levels of S9 mix. Should an increase in reverent colony numbers then be observed which satisfies paragraph (a) the substance is considered to show evidence of mutagenic activity in this test system. If no clear "positive" response can be obtained, the test data may be subjected to analysis to determine the statistical significance of any observed increases in reverent colony numbers, The statistical procedures used will be those described by Mahon *et al* (1989) and will usually be analysis of variance followed by Dunnett's test.

Mahon, G.A.T., Green, M.H.L., Middleton, B., Mitchell, I de G., Robinson, W.D. and Tweats, D.J. (1989). Analysis of data from microbial colony assay in: Kirkland, D.J. (ed.) *UKEMS*

Subcommittee on Guidelines for Mutagenicity Testing. Report Part III. Statistical Evaluation of Mutagenicity Data. P.26. Cambridge University Press. Cambridge.

Positive/Negative Controls: Positive without S9:
N-Ethyl-N'-nitro-N-nitrosoguanidine in DMSO @ 5μg/plate for TA 1535, 3μg/plate for TA 100 and 2μg/plate for CM891

9-Aminoacridine in DMSO @80 μg/plate for TA 1537

2-Nitrofluorene in DMSO @ 1 μg/plate for TA 98

Positive with S9

2-Aminoanthracene in DMSO @ 2μg/plate for TA 1535 and 1 0μg/plate for CM891

Benzo[a] pyrene in DMSO @ 5μg/plate TA 1537, TA 98 and TA 100

Negative
DMSO

Repeat Test: Second test includes a preincubation stage. First test is only the standard plate incorporation.

Results

Cytotoxic Concentrations: No toxicity with or without metabolic activation.

Precipitation Concentration: 5 mg/plate – cloudy solution
1.5 mg/plate – cloudy solution
0.5 mg/plate – slightly cloudy solution
0.15 mg/plate – no observable cloudiness
A slight reduction in cloudiness occurred during the incubation period.

Genotoxic Effects: No genotoxic effects observed with or without metabolic activation.

Conclusion: No evidence of mutagenic activity in this bacterial system.

Data Quality: 1, Reliable without restrictions

References: Benzoflex 2-45. Bacterial Mutation Assay *(S. typhimurium* and *E. coli*). Huntingdon Life Sciences. 1998

MAMMALIAN CELL GENE MUTATION

Test Substance: See boiling point for purity

Solvent: DMSO

Study Type: Nonbacterial mammalian cell gene mutation assay

Test Method: OECD 476

GLP: **Yes**

Year Performed: **1997**

Laboratory: Huntingdon Life Sciences

Species/Strain: Mouse lymphoma L5178Y

Concentrations: 50, 100, 150,200,250, 275, 300, 325, 350 µg/ml

Metabolic Activation: Sprague-Dawley rat liver

Quantity of Activator: 4 ml

Induction: Stimulated by Aroclor 1254

Criteria for Evaluating Results:

Criteria for a positive response:

An increase of at least 100 in the mutant frequency in treated cultures relative to the concurrent control.
The demonstration of a statistically significant increase in mutant frequency following treatment with the test substance.
Evidence of a dose relationship over at least two consecutive dose levels, in any increases in mutation frequency.
Demonstration of reprodicibility in any increase in mutant frequency.
An increase in absolute colony numbers in the treated cultures.
The RTG of cultures showing an increase in mutant frequency should not be less than 10%.

Positive/Negative Controls: Positive without S9:
Methylmethane sulphonate in DMSO
Positive with S9:
20-methylcholanthrene in DMSO

Results

Genotoxic Effects: In the absence of S9-increases in mutant frequency were observed 350 ug/ml on Test 1 and 200 and 325 ug/ml in Test 2. The increases were not 100 above the control level and were within the historical control range. It was concluded that 2-45 did not demonstrate mutagenic potential in the absence of S9 mix. There was no substantive increases in mutant frequency observed in the presence of S9 mix.

Interpretation/conclusion: It is concluded that Benzoflex 2-45 did not demonstrate mutagenic potential in this *in* vitro gene mutation assay

Data Quality: 1, Reliable without restrictions

References: Benzoflex 2-45. Mammalian Cell Mutation Assay. Huntingdon Life Sciences. 1998.

MAMMALIAN CHROMOSOME ABERRATION TEST

Test Substance: See boiling point for purity

Solvent: DMSO

Study Type: *In-vitro* Mammalian Chromosome Aberration Test in CHL cells

Test Method: **OECD 473**

GLP: **Yes**

Year Performed: 1997

Laboratory: Huntingdon Life Sciences

Species/Strain: Chinese Hamster Lung, strain JCRB0030

Concentrations: 4.9, 9.8, 19.5, 39.1, 78.1, 156.3, 312.5 and 625 μg/ml

Metabolic Activation: Sprague-Dawley rat liver

Quantity of Activator: 1.25 and 5 ml

Induction: Stimulated by Aroclor 1254

Criteria for Evaluating Results:

Aberrations were scored according to the classification of ISCN (1985). An International System for Human Cytogenetic Nomenclature, Harden, DG and Klinger, HP (Eds). S. Karger AG. Basel

Positive/Negative Controls: Positive without S9:
Mitomycin C in sterile deionized water
Positive with S9:
Cyclophosphamide in sterile deionized water

Results

Genotoxic Effects: No statistically significant increases in the proportion of aberrant cells, when compared to the solvent control, were seen in either the presence or the absence of S9 mix. A small response seen in the first test, with S9 mix, was not reproduced in the repeat test or at the later harvest. This response was not considered to be indicative of clastogenic activity.

Data Quality: 1, Reliable without restrictions

References: Benzoflex 2-45. *In-vitro* Mammalian Chromosome Aberration Test in CHL cells. Huntingdon Life Sciences. 1998.

ADDITIONAL STUDIES

Benzoflex 2-45. Skin Sensitization to the Guinea Pig. Huntingdon Life Sciences. 1998. OECD 406. Benzoflex 2-45 did not produce evidence of skin sensitization (delayed contact hypersensitivity) in any of twenty test animals. Evidence of skin sensitization was produced by hexyl cinnamic aldehyde (HCA) in all ten positive controls thus confirming the sensitivity of the method.

Benzoflex 2-45. Acute Toxicity (LC_{50}) to the Earthworm (*Eisenia foetida)*. Huntingdon Life Sciences. 1998. OECD 207. Under the conditions of this study, the LC_{50} of Benzoflex 2-45 to the earthworm was found to be in excess of 1000 ppm. The NOEL was considered to be 1000 ppm.

Evaluation of Velsicol Benzoflex 2-45 and Benzoflex 9-88 Plasticizers for Estrogenic Activity Using Vaginal Cornification and the Uterotrophic Response in the Ovariectomized Adult Rat as the Endpoints. BIOQUAL, Inc. 1997. Benzoflex 2-45 did not induce vaginal cornification at doses of 500, 1000, 1500 or 2000 mg/kg/day for 7 days by oral gavage in ovariectomized adult Spraque-Dawley (CD) rats. Benzoflex 2-45 did not stimulate a uterine weight increase or an increase in the uterine weight to final body weight ratio at doses of 500, 1000, 1500 or 2000 mg/kg/day for 7 days. When compared with the vehicle control (corn oil) and positive control (diethylstilbestrol), these data demonstrate that Benzoflex 2-45 did not exhibit estrogenic activity up to and including the maximally tolerated dose.

Benzoflex 2-45. Metabolism in the Rat. Huntingdon Life Sciences. 2000. OECD 417. The metabolism of diethylene glycol dibenzoate was studied after both single oral low level (50 mg/kg) and high level (750 mg/kg) doses to groups of 4 male and 4 female rats. The tissue distribution of radioactivity was studied after low-level doses. The proportions and nature of metabolites were also investigated. Virtually all of single oral doses of 50 and 750 mg/kg of Benzoflex 2-45 administered to Sprague-Dawley CD rats were adsorbed, metabolized and excreted in the urine within 24 hours of administration. Benzoflex 2-45 was metabolized via hydrolysis of the ester bonds to benzoic acid; this free acid was then conjugated with either glycine (major pathway) or glucuronic acid (minor pathway) prior to excretion. The study was conducted to GLP, to fulfil OECD guideline 417 and EU guideline 88/302/EEC (Part B).