REACH

Oxydiethylene dibenzoate

EC number: 204-407-6 | CAS number: 120-55-8

Toxicological information

Repeated dose toxicity: oral

001 Key | Experimental result

Administrative data

Endpoint:	sub-chronic toxicity: oral
Type of information:	experimental study
Adequacy of study:	key study
Study period:	12 May 1997 - 10 September 1997
Reliability:	1 (reliable without restriction)
Rationale for reliability incl. deficiencies:	other: The study was conducted in accordance with OECD and Japanese test guidelines, and in compliance with GLP.

Data source

Reference

Reference Type:	study report
Title:	Unnamed
Year:	1999
Report date:	1999

Materials and methods

Test guideline

Test guideline 1

Qualifier:	according to guideline
Guideline:	OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity Study in Rodents)
Deviations:	no

Test guideline 2

Qualifier:	according to guideline
Guideline:	other: Toxicity test guidelines published in Notification Yakushin 1 No. 24 of the Pharmaceutical Affairs Bureau, Japanese Ministry of Health and Welfare, dated 11 September 1989.
Deviations:	no

GLP compliance:	yes
Limit test:	no

Test material

Test material information

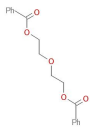
Constituent 1

Reference substance name:	Benzoflex 2-45
IUPAC Name:	Benzoflex 2-45

Constituent 2

Reference substance name:	Diethylene glycol dibenzoate (technical)
IUPAC Name:	Diethylene glycol dibenzoate (technical)

Constituent 3



Reference substance name:	Oxydiethylene dibenzoate
EC Number:	204-407-6
EC Name:	Oxydiethylene dibenzoate
Cas Number:	120-55-8
Molecular formula:	C18H18O5
IUPAC Name:	2-[2-(benzoyloxy)ethoxy]ethyl benzoate

Details on test material:

- Name of test material (as cited in study report): Benzoflex 2-45 (& diethylene glycol dibenzoate)
- Physical state: A solid (warmed up to 40°C to obtain a liquid state)
- Analytical purity: 89.9% (w/w). No correction made for purity.
- Impurities (identity and concentrations):
. Diethylene glycol monobenzoate: 5.85% (w/w0
. Dipropylene glycol dibenzoate: 1.50% (w/w0
. Ethylene glycol dibenzoate: 0.19% (w/w0
. Unidentified component: 0.54% (w/w)
. Unidentified component: 0.10% (w/w)
. Unidentified component: 0.10% (w/w)
- Lot/batch No.: 56625030
- Expiration date of the lot/batch: September 1998, over 1 year from last analysis
- Stability under test conditions: Formulations of the substance were shown to be stable for up to 22 days at ambient temperature.
- Storage condition of test material: Room temperature.

Test animals

Species:	rat
Strain:	other: Crl:(IGS) CD BR
Sex:	male/female
Details on test animals or test system and environmental conditions:	TEST ANIMALS - Source: Charles Rives Breeding Laboratories, Manston Road, Margate, Kent, UK. - Age at study initiation: 7 to 8 weeks - Weight at study initiation: 231 to 301 g for males, 161 to 213 g for females. - Housing: Housed in suspended cages with wire mesh floors, 5 rats of the same sex per cage. Each cage measured 25.7 cm high, 35.8 cm wide, and 53 cm in depth. - Diet (e.g. ad libitum): Free access to ground SDS Rat and Mouse No.1 maintenance diet - Water (e.g. ad libitum): Free access to tap water. - Acclimation period: 19 days from receipt of animals until allocation to groups; a further 7 days from allocation until the commencement of dosing. ENVIRONMENTAL CONDITIONS - Temperature (°C): Nominally 21 ± 2°C; actual range 16 - 28°C - Humidity (%): Nominally 55 ± 10%; actual range 42 - 80% - Photoperiod (hrs dark / hrs light): 12 hours darkness / 12 hours light per 24 hour period. IN-LIFE DATES: From: 16 April 1997 (Animal arrival) / 12 May 1997 (First day of dosing) To: 14 August 1997 (Main Kill) or 10 September 1997 (Recovery kill)

Administration / exposure

Route of administration:	oral: feed
Vehicle:	other: SDS Rat and Mouse No. 1 maintenance diet
Details on oral exposure:	DIET PREPARATION - Rate of preparation of diet (frequency): Weekly - Mixing appropriate amounts with (Type of food): SDS Rat and Mouse No. 1 maintenance diet - Storage temperature of food: Room temperature
Analytical verification of doses or concentrations:	yes
Details on analytical verification of doses or	At weeks 1 and 11 a representative sample of the dietary formulation at each dose level was obtained, sub-sampled,

concentrations:	extracted (soxhlet extraction in acetone, then diluted in acetone as appropriate, then evaporated to dryness using RFE, and re-dissolved in HPLC mobile phase), then analysed by HPLC-UV. Prior to the first analysis, the stability and homogeneity of the formulations was confirmed at nominal concentrations 50 ppm and 60000 ppm for up to 22 days at ambient temperature. The analytical method was validated before use with respect to linearity of detector response, precision of injection, specificity, limit of detection, and accuracy and precision of the extraction technique (procedural recovery). Mean concentrations for DEGDB in test diet formulations analysed during the study were within 7% of nominal concentrations, confirming the accuracy of formulation.
Duration of treatment / exposure:	13 weeks. Selected animals in the control and high dosage levels were maintained for a 4 week recovery period to monitor the reversibility of any treatment-related effects.
Frequency of treatment:	Continuous (the test material was administered in the diet, which was available ad liitum)

Doses / concentrations

Remarks:	Doses / Concentrations: 250, 1000, 1750, 2500 mg/kg/day Basis: other: The concentrations of test substance in diet were changed each week to preserve the required dosage levels for each group.

No. of animals per sex per dose:	10, 20 for control and high level groups to account for recovery animals.
Control animals:	yes, plain diet
Details on study design:	- Dose selection rationale: Treatment levels were selected by the Sponsor on the basis of available toxicity data specifically a preliminary toxicity study performed at this laboratory. - Rationale for animal assignment (if not random): Not applicable - random

Examinations

Observations and examinations performed and frequency:	CAGE SIDE OBSERVATIONS: Yes - Time schedule: At least once daily - Animals were observed for behavioural changes, reaction to treatment, or signs of ill health DETAILED CLINICAL OBSERVATIONS: Yes - Time schedule: Daily BODY WEIGHT: Yes - Time schedule for examinations: On the day of allocation to groups, then once a week thereafter. FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study): - Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: No - food consumption per cage and per group was calculated for each week. - Compound intake calculated as time-weighted averages from the consumption and body weight gain data: Yes FOOD EFFICIENCY: - Body weight gain in kg/food consumption in kg per unit time X 100 calculated as time-weighted averages from the consumption and body weight gain data: No - a group mean value for the food convertion ratio was calculated. WATER CONSUMPTION: Yes - Time schedule for examinations: Monitored visually daily throughout the study. Water consumption was measured accurately, by weight, over daily periods during week 12 of the study. OPHTHALMOSCOPIC EXAMINATION: Yes - Time schedule for examinations: Examined before commencement of daose, and during week 13 of dosing. - Dose groups that were examined: All rats examined before dosing; all rats in groups 1 and 5 (control and 2500 mg/kg/day) examined at week 13 HAEMATOLOGY: Yes - Time schedule for collection of blood: Samples taken from all animals in week 13, and for all recovery animals in recovery week 4. - Anaesthetic used for blood collection: Yes (ether)

	- Animals fasted: Yes - How many animals: All animals - Parameters checked: Packed cell volume, Haemoglobin, Red cell count, Mean corpuscular haemoglobin concentration, Mean corpuscular volume, Total white blood cell count, Differential white blood cell count, Neutrophils, Lymphocytes, Eosinophils, Basophils, Monocytes, Large unstained cells, Cell morphology, Platelet count, Reticulocyte count, Prothrombin time, Activated partial thromboplastin time, and Thrombotest. CLINICAL CHEMISTRY: Yes - Time schedule for collection of blood: Samples taken from all animals in week 13, and for all recovery animals in recovery week 4. - Animals fasted: Yes - How many animals: All animals - Parameters checked: Total protein, Glucose, Urea nitrogen, Creatinine, Alkaline phosphatase, Glutamic-pyruvic transaminase, Glutamic-oxolacetic transaminase, Gamma-glutamyltransferase, Sodium, Potassium, Calcium, Chloride, Inorganic phosphorous, Cholesterol, Protein electrphoresis (Albumin, α1-Globulin, α2-Globulin, β-Globulin, γ-Globulin, Total globulin), A/G ratio, Ornithine carbamoyl transferase, and Triglycerides. URINALYSIS: Yes - Time schedule for collection of urine: Samples taken from all animals in week 13, and for all recovery animals in recovery week 4 - Metabolism cages used for collection of urine: No data - Animals fasted: Yes - Parameters checked: Volume, pH, Specific grvity, Protein, Urinary sodium, Urinary potassium, Urinary chloride, Total reducing substances, Glucose, Ketones, Bile pigments, Urobilinogen, Haem pigments, and Microscopy.
Sacrifice and pathology:	GROSS PATHOLOGY: Yes (See table 1, below) HISTOPATHOLOGY: Yes
Statistics:	The following sequence of statistical tests was used for food consumption water consumption bodyweight clinical pathology and organ weight data: If the data consisted predominantly of one particular value (relative frequency of the mode exceeded 75%) the proportion of animals with values different from the mode was analysed (Fisher 1950 and Mantel 1963) Otherwise: A test was applied to test for heterogeneity of variance between treatments (Bartlett 1937) Where significant (at the 1% level) heterogeneity was found a logarithmic transformation was tried to see if a more stable variance structure could be obtained. If no significant heterogeneity was detected or ifa satisfactory transformation was found a one way analysis ofvariance was carried out If significant heterogeneity of variance was present and could not be removed by a transformation an analysis of ranks was used (Kruskal and Wallis 1952/3). Analyses of variance were followed by Student's t test and Williams test (1971/2) for a dose related response although only the one thought most appropriate for the response pattern observed was reported. The Kruskal Wallis analyses were followed by the non parametric equivalents of these tests (Shirley 1977). Where appropriate analysis of covariance was used in place of analysis of variance in the above sequence For organ weight data analysis of variance was performed using terminal bodyweight as covariate when the within group relationship between organ weight and bodyweight was significant at the 10% level in an attempt to allow for differences in bodyweight which may have influenced the organ weights.

Results and discussion

Results of examinations

Clinical signs:	effects observed, treatment-related
Mortality:	mortality observed, treatment-related
Body weight and weight changes:	effects observed, treatment-related
Food consumption and compound intake (if feeding study):	effects observed, treatment-related
Food efficiency:	effects observed, treatment-related
Water consumption and compound intake (if drinking	no effects observed

water study):	
Ophthalmological findings:	no effects observed
Haematological findings:	effects observed, treatment-related
Clinical biochemistry findings:	effects observed, treatment-related
Urinalysis findings:	effects observed, treatment-related
Behaviour (functional findings):	not examined
Organ weight findings including organ / body weight ratios:	effects observed, treatment-related
Gross pathological findings:	effects observed, treatment-related
Histopathological findings: non-neoplastic:	effects observed, treatment-related
Histopathological findings: neoplastic:	not examined
Details on results:	CLINICAL SIGNS AND MORTALITY One male receiving 2500 mg/kg/day was sacrificed in Week 4 due to poor clinical condition following 2 convulsive like episodes. Another male receiving 2500 mg/kg/day showed a similar episode on a single occasion but fully recovered. A hunched posture and body tremors were noted during the treatment period principally in rats receiving 2500 mg/kg/day. These findings were considered to be related to the stress associated with treatment. BODY WEIGHT AND WEIGHT GAIN Bodyweight gain fluctuated in all treated groups but was reduced to a toxicologically significant degree only at 1750 or 2500 mg/kg/day. An adverse palatability reaction in Weeks 1 to 2 was insufficient to fully account for these changes. Weight gain for animals previously receiving 2500 mg/kg day was vastly superior to that of Controls in the Recovery period such that the intergroup deficit was almost offset within 4 weeks. FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study) There appeared to be an adverse palatibility reaction over the first few days of treatment, with 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 I 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. FOOD EFFICIENCY The efficiency of food (utilisation assessed by calculation of the food conversion ratios (FCRs)) was below that of Controls for both sexes receiving 2500 mg/kg/day throughout the treatment period and for both sexes receiving 1750 mg/kg/day during the latter half of the treatment period. During the Recovery period the efficiency of food utilisation for both sexes previously treated at 2500 mg/kg/day was vastly superior to that of the controls reflecting the improved bodyweight performance at that time. HAEMATOLOGY 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 results were characteristic for rats of this age. The only notable individual finding was an apparent anaemia in two rats at 2500 mg/kg/day, characterised by low PCV haemoglobin erythrocyte count and mean corpuscular haemoglobin concentrations together with a high reticulocyte count indicative of a regenerative anaemia. This 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 (PT, TT and APTT) were normal for rats of this age and strain therefore the minor intergroup differences are considered to be coincidental and of no toxicological importance. A single animal at 2500 mg/kg/day 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.
By Week 4 of the Recovery period the regenerative anaemia previously noted in the two rats at 2500 mg/kg/day had fully recovered. All other group
mean and individual values were considered to be characteristic for rats of this age despite the few statistically significant intergroup differences.

CLINICAL CHEMISTRY
Plasma glucose levels at Week 13 were towards or below the lower end ofthe nonnal 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 three animals 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.

URINALYSIS
The urine of treated animals showed a dosage related increase in acidity and sodium potassium concentrations were reduced in both sexes receiving 1750 or 2500 mg/kg/day which may be associated with excretion of acidic metabolites.

ORGAN WEIGHTS
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 be indicative of complete recovery. The increased liver weight at the highest doses correlated with the observation ofminimal liver cell hypertrophy.
Although group mean spleen weight adjusted for terminal bodyweight was increased to a statistically significant degree for females receiving 1000
mg/kg/day or above this finding cannot be conclusively attributed to treatment particularly as there was no similar trend in absolute spleen weight in male treated groups and as the histopathological findings in the spleen could not wholly account for the change in the weight of this organ at the
Main kill.

HISTOPATHOLOGY: NON-NEOPLASTIC
Liver - Periportal hepatocyte hypertrophy (minimal in degree) 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 adjusted and as a percentage of terminal
bodyweight for males and females receiving 2500 mg/kg/day and the increased values recorded for liver transaminases for male rats receiving
2500 mg/kg/day.
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.

Effect levels

Dose descriptor:	NOEL
Effect level:	1 000 mg/kg bw/day (nominal)
Sex:	male/female
Basis for effect level:	other: overall effects

Target system / organ toxicity

Critical effects observed:	not specified

Applicant's summary and conclusion

Conclusions:	There were no findings of toxicological importance at a dosage of 1000 mg/kgbw/day or below. When selected animals previously receiving 2500 mg/kgbw/day were maintained off-dose for 4 weeks, all treatment-related changes showed evidence of, or complete recovery.
Executive summary:	A 13 -week repeated oral dose (dietary) study was conducted to determine the effects of prolonged exposure on rats of the test material DEGDB. The study was conducted according to OECD and Japanese test guidelines, and in compliance with GLP. Groups of five rats (of each sex) were dosed by dietary administration with DEGDB for a period of 13 weeks at levels 0 (untreated diet control), 250, 1000, 1750, and 2500 mg/kgbw/day. Additional rats were dosed at 0 and 2500 mg/kgbw/day to allow for an assessment of recovery from treatment for four weeks after dosing. No findings of toxicological importance were detected in this study at a dosage of 1000 mg/kgbw/day or below. Dosages of 1750 or 2500 mg/kgbw/day were tolerated (with one exception - a single mortality at 2500 mg/kg/day) but induced clinical findings changes in blood parameters, minor treatment-related pathology and/or adverse effects on bodyweight gain. When selected animals previously receiving 2500 mg/kgbw/day were maintained off dose for 4 weeks all treatment related changes showed evidence of or complete recovery. The NOEL was 1000 mg/kgbw/day.