REACH

Oxydipropyl dibenzoate

EC number: 248-258-5 | CAS number: 27138-31-4

Toxicological information

Repeated dose toxicity: oral

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:	guideline study

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

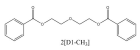
Test material

Test material information

Constituent 1

Reference substance name:	Benzoflex 9-88
IUPAC Name:	Benzoflex 9-88

Constituent 2



Reference substance name:	Oxydipropyl dibenzoate
EC Number:	248-258-5
EC Name:	Oxydipropyl dibenzoate
Cas Number:	27138-31-4
Molecular formula:	C20H22O5
IUPAC Name:	oxydipropyl dibenzoate

Details on test material:
- Name of test material (as cited in study report): Benzoflex 9-88 (Dipropylene glycol dibenzoate DPGDB)
- Physical state: Clear colourless liquid
- Stability under test conditions: 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 River Breeding Laboratories, Manston Road, Margate, Kent, UK - Age at study initiation: 7 to 8 weeks - Weight at study initiation: 220 to 297 g for males and 159 to 208 g for females. - Housing: Housed in suspended cages with wire mesh floors, 5 rats of the same sex per cage. Each cage measures 25.7 cm high, 35.8 cm wide and 53 cm in depth. - Diet: Free access to ground SDS Rat and Mouse No 1 maintenance diet - Water: 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 17.5-28°C - Humidity (%): Nominally 55 ± 10%;, actual range 38-74% - Photoperiod: 12 hours darkness / 12 hrs light per 24 hour period IN-LIFE DATES: From: 16 April 1997 (Animal arrival) / 12 May 1997 (First day of dosing) To: 11- 14 August 1997 (Main Kill) or 9 - 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 concentrations:	At weeks 1 and 11 a representative sample of the dietary formulation at each dose level was obtained, sub-sampled, 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 DPGDB in test diet formulations analysed during the study were within 10% 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

Doses / concentrations 1

Remarks:	Doses / Concentrations: 250 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.

Doses / concentrations 2

Remarks:	Doses / Concentrations: 1000 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.

Doses / concentrations 3

Remarks:	Doses / Concentrations: 1750 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.

Doses / concentrations 4

Remarks:	Doses / Concentrations: 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. - Rationale for animal assignment (if not random): Not applicable - random
Positive control:	None

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 dose, 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:	no effects observed
Mortality:	no mortality observed
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 water study):	not specified
Ophthalmological findings:	effects observed, treatment-related
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 There were no unscheduled deaths. There were no findings which could be conclusively related to treatment. Incidental findings of hairloss and stained fur were noted, however the incidence showed no correlation to dosage and/or these findings were also noted in the Control group. BODY WEIGHT AND WEIGHT GAIN There was a general dosage-related adverse effect on group mean bodyweight gain in both sexes. With a few exceptions (females receiving 250 mg/kg/day in Week 1, males treated with 1000 mg/kg/day Weeks 6 to 13) this was generally evident throughout the dosing period. However in rats treated with 1000 mg/kg/day or below the degree of change was insufficient to be of toxicological importance. This is supported by the fact that final bodyweights for males and females at 1000 mg/kg/day were only 5% less than controls. The degree of suppression of weight gain (-14%) in females receiving 1000 mg/kg/day exceeded that of males at this dosage (-8%) and achieved statistical significance. However this finding is considered unlikely to be of toxicological importance as data from higher dose groups indicate that males were more likely to be susceptible to treatment-related adverse effect on bodyweight. Small reductions in food intake were insufficient to fully account for the adverse effect on bodyweight. During the 4-week Recovery period, animals previously receiving 2500 mg/kg/day showed vastly superior weight gain than Controls, however 4 weeks appeared to be an insufficient time to completely rectify the effects of 13 weeks treatment. FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study) An overall slight but dosage related reduction in group mean food consumption was noted for males receiving 1750 or 2500 mg/kg/day. In addition a slight reduction in group mean food intake was noted for females receiving 2500 mg/kg/day principally during Week 1 of treatment. The quantities of spilt diet recorded indicated that there may have been a slight adverse palatability reaction in these groups particularly during Week 1. Consumption by all other groups and by rats previously receiving 2500 mg/kg/day during the Recovery period was considered to be comparable to that of Controls. FOOD EFFICIENCY As treatment-related effects bodyweight were more pronounced than those on food consumption, trends in the efficiency of food utilisation generally reflected the bodyweight change, namely; With a few exceptions there was a general dosage-related adverse effect of treatment on the efficiency of food utilisation in both sexes. The degree of change was however small at the lower dosages and was considered to be of toxicological importance only in both sexes receiving 1750 or 2500 mg/kg/day.

During the Recovery period the efficiency of food utilisation for both sexes previously treated at 2500 mg/kg/day was superior to that of the controls, reflecting the improved bodyweight performance at that time.

OPHTHALMOSCOPIC EXAMINATION
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. Ophthalmoscopy was therefore not performed during the Recovery period.

HAEMATOLOGY
Several parameters at Week 13 achieved levels of statistical significance as detailed below, however none could be conclusively related to treatment.
A number of erythrocyte parameters achieved levels of statistical significance in Week 13 for rats receiving 1000 mg/kg/day or above (increased red cell counts, reduced mean corpuscular volume and reduced mean corpuscular haemoglobin). There were, however, few indications of a dosage-relationship and most individual values were either within the range encountered in Controls or were generally characteristic of rats of this age (within the range of background data at this laboratory). These minor intergroup differences are therefore considered not to be of toxicological importance.
Similarly, levels of statistical significance were achieved for clotting tests (PT, IT and APTT) in one or both sexes at 1000 mg/kg/day or above. The only indication of a dosage relationship was for a reduction in male TT and APTT data, but with the exception of a low TT for Rats 43M (1750 mg/kg/day)and 60M (2500 mg/kg/day), all individual values were characteristic of this species (and the group mean data for treated females generally exceeded that of the Controls). Intergroup differences in clotting tests are therefore considered to be coincidental.
Individual leukocyte data for males receiving 2500 mg/kg/day at Week 13 were generally towards the lower end ofthe normal range. Although statistical significance was achieved for group mean values, the lowest single result at Week 13 was for a Control animal (Rat 135M). In addition, group meanleukocyte data of females receiving 2500 mg/kg/day generally exceeded that of Controls. The slightly lower group mean results for males therefore cannot be conclusively attributed to treatment.
There were no other notable findings at Week 13 and all data at Recovery Week 4 was unremarkable.

CLINICAL CHEMISTRY
Plasma AP activity was very high at Week 13 for 4 rats receiving 2500 mg/kg/day and slightly raised for 6 males receiving 2500 mg/kg/day and 3 ratstreated with 1750 mg/kg/day. Levels of statistical significance were achieved for male group mean values at 1750 or 2500 mg/kg/day. By Recovery Week 4 all AP activities of surviving animals had returned to a level comparable to that of Controls.
There was no similar effect of treatment in females. All individual female AP activities were characteristic of the species and those in treated groups were generally comparable to the range encountered in the Controls. The level of statistical significance achieved for female group mean values at Week 13 is therefore coincidental. Female untreated rats are more prone to exhibit spontaneously raised plasma GPT, GOT and OCT, activities than their male counterparts. In addition rats only slightly older than those employed in this study sometimes show sporadic and unexplained high transaminase activities.
In Recovery Week 4 the only notable plasma enzyme activities were in Rat 117F previously receiving 2500 mg/kg/day In isolation and as spontaneous high individual activities are not unknown this finding is considered likely to be coincidental. All other animals previously receiving 2500 mg/kg/day had enzyme activities comparable to concurrent Controls indicating complete reversibility of the treatment related changes. Group mean plasma cholesterol was increased to a statistically significant degree in males receiving 1000 mg/kg/day or above but without a dosage relationship. The group mean cholesterol level of females treated with 2500 mg/kg/day was slightly in excess of Controls and achieved statistical significance however in the absence of a dosage relationship this was considered not to be of toxicological importance In Recovery Week 4 all cholesterol levels were considered normal.
No other findings were considered to be of toxicological importance

URINALYSIS
The following treatment related findings were noted, however not all individuals were affected. In the absence of renal pathology these are considered to be of little toxicological importance and may represent alteration in renal function and/or be related to excretion of the test material or its metabolites:
Group mean urinary volume was reduced and specific gravity increased at Week 13 for males receiving 1750 or 2500 mg/kg/day with a dosage-relationship however there was no indication of a similar effect in females.
There was a dosage-related effect upon group mean urinary pH of both sexes receiving 1000 mg/kg/day or above at Week 13 with the urine of treated animals being increasingly acidic at

higher dosages levels.
Group mean urinary sodium and potassium concentrations were reduced in Week 13 for both sexes treated with 1750 or 2500 mg/kg/day with results generally achieving statistical significance and showing a dosage relationship in these groups.
By Recovery Week 4, results for animals previously treated with 2500 mg/kg/day were considered comparable to Controls. There were no other notable findings. Intergroup differences in urinary protein and chloride levels were considered of no toxicological importance as all individual results were unremarkable.

ORGAN WEIGHTS
There was a slight treatment related increase in liver weight for females receiving 1750 mg/kg/day and males treated with 2500 mg/kg/day. Females receiving 2500 mg/kg/day showed a more marked increase in liver weight. These changes may be partially associated with increased plasma transaminase levels and the low grade hepatic hypertrophy detected microscopically however there was little consisitency between these data, the degree of hepatic toxicity is considered to be low.

HISTOPATHOLOGY: NON-NEOPLASTIC
Liver - Periportal hepatocyte hypertrophy (dose related) was seen in the males and female rats receiving 1750 or 2500 mg/kg/day. This finding is associated with the increased group mean liver weight adjusted for bodyweight recorded for male and female rats receiving 2500 mg/kg/day and females receiving 1750 mg/kg/day. This microscopic finding was also associated with the increased group mean values recorded for liver transaminases in this treatment group. The Recovery group animals demonstrated that this was a reversible effect.
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 the slight degree of haemosiderosis seen in both male and female rats receiving 2500 and in a smal number of female rats receiving 1750 mg/kg/day was considered to be a treatment related exacerbation of this physiological change. The Recovery group animals demonstrated that this was a reversible finding.
Caecum - An increased incidence of minimal epithelial hyperplasia was seen in male and female rats receiving 2500 mg/kg/day compared to controls. The Recovery group animals demonstrated that this was a reversible effect.

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:	1000 mg/kg/day is considered to represent a non-toxic dose in both sexes. Dosages of 1750 or 2500 mg/kg/day were tolerated but induced changes in blood parameters, minor treatment related pathology and/or adverse effects on bodyweight gain. 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.
Executive summary:	A 13 -week repeated oral dose (dietary) study was conducted to determine the effects of prolonged exposure to rats of the test material DPGDB. 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 4 weeks after the end of dosing. Clinical observations showed no signs of adverse affects with the exception of a decrease in bodyweight gain and final bodyweight in both sexes at the higher doses. The major toxicological findings were limited to the liver, spleen and

caecum at the 1750 and/or 2500 mg/kg/day doses. The liver showed minimal to slight liver cell hypertrophy (enlargement) and alterations in associated biochemical parameters. At 1000 mg/kgbw/day there was a slight increase in liver enzymes but not judged to be sufficient for toxicity. The spleen showed a slight to moderate increase in the normal degree of haemosiderosis (iron accumulation) and the caecum showed a minimal epithelial hyperplasia only at 2500 mg/kg/day. Urinary pH was decreased in a dose related manner in both sexes which was likely due to acidic metabolites being excreted in the urine and may have been related to the decreased elimination of sodium and potassium at the 1750 and 2500 mg/kg/day doses only. Most importantly all treatment related affects were reversible or showed tendency to reverse in the 2500 mg/kgbw/day day 4 week recovery group.

No findings of toxicological importance were detected in this study at a dosage of 1000 mg/kgbw/day or below. The NOAEL was 1000 mg/kgbw/day.