Summaries of Toxicity Studies on Benfuresate

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DESCRIPTION OF THE TEST CHEMICAL

Benfuresate is the active ingredient of corresponding commercially available herbicide formulation. It has been synthesized by Schering AG in 1970s during, and found that the compound shows excellent herbicidal activities to Cyperaceae weeds, such as *Cyperus rotundas* and *Cyperus esculentus*. In Japan, this compound has been investigated since 1987 for the herbicidal activities, and it is registered to control *Eleocharis kuroguwai* in paddy field which is difficult to control with existing herbicides.

To provide an adequate data basis for an evaluation of health risks for users of crop protection agents containing benfuresate and for an assessment of the health risk for consumers of treated food products, numerous toxicological studies with the technical product and 2% granular formulation were conducted.

The chemical structure and physico-chemical properties of benfuresate are given below.

Common name: Benfuresate (BSI/ISO)
Chemical name: 2,3-Dihydro-3,3-dimethylbenzofuran-5-yl ethanesulfonate

Structural formula:

![Structural formula](image)

Molecular formula: C_{12}H_{16}O_{4}S
Appearance: Brown viscous liquid which may solidify at room temperature
Boiling point: 239-242°C (atmospheric pressure)
Vapour pressure: 1.4 x 10^{-3} (20°C)
Solubility (25°C): Water 0.261 g/l. Soluble in methanol, acetone, dichloromethane, toluene and ethyl acetate
Partition coefficient (log \( P_{ow} \)): 2.41
Stability: Stable for 31 days under pH 5, 7 and 9 in 25°C and 37°C
Photolysis: Half-life time is about 7 days for aqueous solution

ACUTE TOXICITY STUDIES

The results of acute toxicity studies are summarized in Table 1.

1. Acute Oral and Dermal Toxicity Study

On oral administration, benfuresate technical material and 2% granule formulation
Table 1  Acute toxicity test.

<table>
<thead>
<tr>
<th>Route</th>
<th>Species</th>
<th>LD_{50} (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td>Rat^{a)}</td>
<td>M, F &gt;4000</td>
</tr>
<tr>
<td></td>
<td>Mouse^{b)}</td>
<td>M, F &gt;5000</td>
</tr>
<tr>
<td>Dermal</td>
<td>Rat^{c)}</td>
<td>M, F &gt;5000</td>
</tr>
<tr>
<td>Technical</td>
<td>(4hr. LC_{50})</td>
<td>M, F &gt;5.343 (mg/l)</td>
</tr>
<tr>
<td>Inhalation</td>
<td>Rat^{d)}</td>
<td>M, F &gt;5000</td>
</tr>
<tr>
<td>Formulation</td>
<td>Oral</td>
<td>M, F &gt;5000</td>
</tr>
<tr>
<td>(2% granule)</td>
<td>Mouse^{f)}</td>
<td>M, F &gt;5000</td>
</tr>
<tr>
<td>Code: NS 112</td>
<td>Dermal</td>
<td>M, F &gt;2000</td>
</tr>
</tbody>
</table>

\^{a-e)} AgrEvo UK Ltd., 1991.  
\^{d} Hazleton UK, 1990.  
\^{e-f)} Huntingdon Research Centre, 1992.  
M : male, F : female.

is unlikely to indicate particular toxic signs in rats and mice.
No harmful skin reactions nor toxic signs by dermal toxicity study with either technical or formulation were observed.

2. Acute Inhalation Toxicity Study

One group of ten rats (five males and five females) of the Crl: CD(SD)BR strain was exposed to technical benfuresate at a single chamber concentration of 5.343 mg/l by inhalation (head-only) over a period of 4 hr. The corresponding nominal concentration was 6.455 mg/l. A group of ten rats (five males and five females) was similarly exposed to filtered air as a control. Exposure was followed by an observation period of 14 days.

The exposure chamber temperature recorded for both the control and treated groups was 19°C, and the chamber relative humidity was in the range 26 to 58%. The chamber air flow rates were 20.0 and 14.2 l/min for the control and treated group. The mean mass median aerodynamic diameter of the particles in the atmosphere was 1.73 μm.

There were no deaths during the course of the study. Treatment-related clinical signs occurring during the day of exposure included piloerection, salivation, nasal secretion and coldness to touch. Clinical signs did not persist beyond the day of exposure. There were no treatment related effects on body weight or absolute and relative lung weights and no evidence of specific target organ toxicity.
IRRITATION STUDIES

1. Primary Dermal Irritation Study on Rabbits

A skin irritation potential of technical benfuresate was evaluated according to OECD guideline 404 for the testing of chemicals, and a skin irritation of 2% granule was assessed according to the Japanese testing guideline for primary dermal irritation.

0.5 ml of technical benfuresate or 0.5 g of 2% granule were applied under a 2.5 cm square gauze pad to the intact skin of a shaved area. Each treatment site was covered with "Elastoplast" elastic adhesive dressing for a four period.

The skin was grossly examined 30 to 60 min after the exposure period and then daily for 3 days on technical benfuresate and for 4 days on 2010 granule.

No signs of irritancy were observed following a single-semi-occlusive application of technical benfuresate or 2% granule to intact rabbit skin for 4 hr.

(Technical: Schering Agrochemicals Ltd., 1990)
(Granule: Huntingdon Research Centre, 1992)

2. Primary Eye Irritation Study on Rabbits

Evaluation of eye irritation properties of technical benfuresate and 2% granule was made according to OECD guidelines for the testing of eye irritancy. 0.1 ml of technical benfuresate and approximately 100 mg of 2% granule, the weight occupying a volume of 0.1 ml were placed into the lower lid of one eye of each animal. The eyelids were then gently held together for one second before releasing. The contralateral eye remained untreated and served as a control. The eyes were grossly examined 1 hr after the exposure period and then 1, 2, 3 and 4 days after instillation for technical benfuresate and 7 days was included for 2% granule.

There were no signs of irritation seen in any animal.

(Technical: Schering Agrochemicals Ltd., 1990)
(Granule: Huntingdon Research Centre, 1992)

3. Dermal Sensitization Study on Guinea Pigs

The potential of technical benfuresate and the 2% granule to induce delayed contact hypersensitivity were evaluated according to the Buehler test.

Induction comprised topical occlusive exposure of 10 female albino guinea pigs of Dunkin/Hartley strain to undiluted benfuresate and suspension of 70% w/w of 2% granule in distilled water for 6 hr, once per week for 3 weeks. Treated and naive Guinea pigs were challenged 14 days after the last induction dose with undiluted benfuresate and the suspension of 2% Study granule.

Technical benfuresate and 2% granule exhibited no evidence of dermal sensitization potential.

(Technical: Schering Agrochemicals Ltd., 1990)
(Granule: Huntingdon Research Centre, 1992)
SUB-CHRONIC TOXICITY STUDIES

1. Rat 90-Day Dietary Repeat Dose Study

Group of 10 male and 10 female CD(SD) rats were fed diet containing 0, 200, 500, 1250 or 3125 ppm (equivalent to 0, 14, 35, 88 and 218 mg/kg/day) of technical benfuresate for 13 weeks.

There were no effect on clinical signs, body weight, food consumption, water consumption, urinalysis, and haematological parameters, considered to be related to treatment with test article. Kidney weight relative to body weight was increased by 10% in males fed 3125 ppm.

Treatment also caused an increase in the incidence and severity of hyaline droplet degeneration and eosinophilic cytoplasmic inclusion bodies in the proximal tubular epithelium of the kidneys of male rats.

The no adverse effect level following subchronic dietary administration of technical benfuresate to rat for 13 weeks was 1250 ppm, equivalent to 88.2 mg/kg/day.

(Schering Agrochemicals Ltd., 1990)

2. Mouse Dietary 90 Day Study

Groups of 10 male and 10 female mice were fed diet containing 0, 1000, 3000, 9000 or 18,000 ppm (equivalent to 0, 236, 846, 2772 or 5454 mg/kg/day) of technical benfuresate for at least 90 days. Supplementary groups of 10 male and 10 female mice were included at each dose level for blood sampling only.

At 18,000 ppm, there were three treatment-related deaths in males. Body weight gain was reduced by 9 and 7% and food consumption was increased by 20 and 38% in males and females, respectively. Water consumption was also increased by 16% in females. In males, kidney weight was decreased (18%) and relative liver weight increased (16%). Macroscopic effects seen in the kidneys of 4/10 males included swelling, paleness and irregular shape and size. Effects seen in the liver of 1/10 males included depressed lobes. Treatment at 18,000 ppm also caused papillary necrosis, tubular degeneration and luminal dilatation of the proximal tubules in the kidneys of 4/10 males and 1/10 females.

At 9000 ppm, there were two deaths in males. Reduced body weight gain and increased food and water consumption, as well as microscopic kidney changes in 1/10 females, were similar to those seen at 18,000 ppm. A reduction in the incidence of fat deposition in hepatocytes of females was also noted.

At 3000 ppm, reduced body weight gain, increased food consumption and reduced fat deposition in hepatocytes of females were similar to those seen at 9000 ppm.

No treatment-related effects were seen at 1000 ppm. On the basis of results obtained, it was concluded that the no effect level (NOEL) was 1000 ppm, equivalent to a daily intake of 236 mg/kg.

(Schering Agrochemicals Ltd., 1991)
3. Dog 90-Day Repeat Dose Study by Gavage

Groups of 4 male and 4 female beagle dogs were dosed daily by gavage at 0, 10, 100 and 1000 mg/kg bodyweight of technical benfuresate for 90 days.

There were no clinical signs directly attributable to administration of benfuresate, the four deaths observed during the study, and two deaths in the highest dose group were considered to be treatment and others being due to misdosing.

Bodyweight and food consumption were unaffected by treatment. No treatment related effects were detected by clinical examinations, ophthalmoscopy, electrocardiography and laboratory investigations.

Renal effects at 1000 mg/kg were characterised by increased kidney weight, changes in gross appearance at necropsy and histopathological evidence of papillary necrosis and scarring, tubular dilation and interstitial nephritis.

Liver weight was increased in female dogs at 1000 mg/kg, but this was not associated with any histopathological change.

No other treatment related changes were detected at necropsy or by organ weight analysis and histopathological examination. The no effect level for daily dosing of benfuresate to dogs was 100 mg/kg. (Schering Agrochemicals Ltd., 1988)

CHRONIC TOXICITY/ONCOGENICITY STUDIES

1. Combined Dietary Chronic Toxicity and Oncogenicity Study in Rat

Groups of 50 male and 50 female CD (SD) rats were fed diet containing 0, 60, 600 or 6000 ppm (equivalent to 0, 3.07, 30.6 or 318 mg/kg/day) of technical benfuresate for 27 months. In addition, supplementary groups of 20 male and 20 female rats treated at the same dietary levels were killed after 12 months.

The high dose was selected as the predicted maximum tolerated dose (MTD) based on comprehensive range finding information.

Treatment at 6000 ppm resulted in persistently decreased weight gain (10-18%) and food intake (6-8%) in both sexes. Food conversion efficiency was also impaired (17%) in females at this dose level. Bilirubin (total) was variably reduced in both sexes whilst females had slightly lower absolute liver and heart weights and kidney weight relative to body weight was elevated by 14%. Histopathology showed a reduction in the severity and incidence of margination of the cytoplasm of hepatocytes in females and a lower incidence of chronic progressive nephropathy and epithelial hyperplasia of the forestomach in both sexes.

At 600 ppm, overall body weight gain was reduced by 11 and 6% in males and females, respectively. Kidney weight relative to body weight was increased in females by 11% The only other finding attributable to treatment was a reduction in the severity and incidence of margination of the cytoplasm of hepatocytes of females and epithelial hyperplasia of the forestomach in both sexes.

No treatment-related effects were observed at 60 ppm.
It was concluded that benfuresate was not oncogenic in the rat at dose levels up to 6000 ppm (equivalent to a daily intake of 318 mg/kg/day).

Treatment at 6000 ppm resulted in clear evidence of toxicity including a marked reduction in weight gain indicating that a maximum tolerated dose was achieved. The no-effect level was 60 ppm equivalent to 3.07 mg/kg/day.

(Schering Agrochemicals Ltd., 1990)

2. Mouse Dietary Opecogenicity Study

Groups of 50 male and 50 female CD1(CR) BR mice were fed diet containing 0, 300, 3000 or 10,000 ppm (equivalent to 0, 55, 561 or 1989 mg/kg/day) of technical benfuresate for 80 weeks.

In males dosed at 10,000 ppm, there was an earlier onset and higher incidence of mortality. Food consumption was increased (11%). Terminal body weight was markedly and statistically significantly lower (14%) in females which was considered to be partly due to a significantly higher intake (20%) of benfuresate than in the corresponding males. Renal papillary necrosis was observed in 16/50 males and was considered fatal in 13 of these resulting in the increased mortality at this dose level. In females renal papillary necrosis was considered fatal in 4/50 animals. There was also an increase in the incidence of pyelonephritis in both sexes. It is probable that there was a palatability problem due to the very high dose level in females, as up to one third of the apparent food consumption was scattered on the cage floor.

In females treated at 3000 ppm, terminal body weight was statistically significantly lower (11%) than controls and renal papillary necrosis was observed in 3/50 animals. There were no other effects at this dose level.

At 300 ppm, terminal body weight was not statistically significantly different from control. There were no treatment-related histopathological lesions detected in either sex.

Technical benfuresate was not oncogenic in the mouse at very high dose levels up to 10,000 ppm (equivalent to a daily intake of 1989 mg/kg/day).

Treatment at 10,000 ppm resulted in clear evidence of toxicity including a reduction in survival in males and a reduction in body weight (females), renal papillary necrosis and pyelonephritis in both sexes, indicating that a maximum tolerated dose was exceeded.

The overall NOEL was 300 ppm (equivalent to a daily intake of 55 mg/kg/day).

(Schering Agrochemicals Ltd., 1992)

3. Dog 12 Month Gavage Repeat Dose Study

Groups of 4 male and 4 female beagle dogs were dosed daily for 12 months by gavage with 0, 4 or 40 mg/kg of technical benfuresate. Another group of 6 male and 6 female dogs were dosed concurrently at 400 mg/kg/day subsequently reduced to
300 mg/kg/day and then 200 mg/kg (administered twice daily from day 86). The control animals received only 1% methyl cellulose in distilled water.

Oral administration of technical benfuresate to dogs at dose levels of up to 40 mg/kg elicited no adverse effects.

A single dose of 400 mg/kg or 300 mg/kg resulted in tremors, convulsions (with and without relaxation), rapid respiration, salivation and frothing at the mouth. Prostration and reduced activity were also noted. These signs were generally seen within 3 hr of dosing and had regressed within 7 hr.

Following twice daily administration of 200 mg/kg, convulsions were seen in one male prior to being sacrificed in extremis on study day 103. Other signs attributed to treatment included circling behaviour and reduced activity in two animals and tremors in one animal. These signs were generally intermittent and most animals were devoid of treatment-related clinical signs for the rest period of the study. In females, water consumption was increased by 17-27% on 3/4 occasions and a reduction in red cell parameters was noted after 4, 7 and 12 months. Red cell parameters were also decreased at 12 months in males. Very severe papillary necrosis was seen in the kidney of one male and one female had chronic pyelonephritis.

Daily administration of single bolus doses of either 400 or 300 mg/kg were found to exceed the maximum tolerated dose leading to twice daily administration of 200 mg/kg which was tolerated. The target organ was the kidney.

The no observed effect level (NOEL) was 40 mg/kg/day.

(Schering Agrochemicals Ltd., 1992)

REPRODUCTIVE AND TERATOGENICITY STUDIES

1. Rat Multigeneration Study

Technical benfuresate was administered in the diet at concentrations of 0, 60, 600 and 6000 ppm.

After about 10 weeks treatment (maturation period), groups of 30 male and 30 female CD(SD) strain F0 generation rats were paired to produce the F1 litters from which the F1 generation was derived.

The selected F1 generation animals were treated for about 11 weeks and then paired to produce the F2 litters. The study was terminated when these offsprings had reached weaning. The following findings were observed in groups dosed at 600 and 6000 ppm;

Adults: At 6000 ppm, group mean body weight gain of both sexes was reduced during the maturation period in both the F0 and F1 generations. Reductions were about 16 and 13% in males and 16 and 9% in females for the F0 and F1 generations respectively.

During gestation, mean body weight gain was reduced by about 10 and 14% in F0 and F1 generation females respectively.
Relative kidney weights of F0 adults were slightly increased. In F0 males alone, this was associated with an increased incidence of minimal hyaline droplet accumulation in the renal proximal tubular epithelium.

**Litters**: At 6000 ppm, in both litterings, group mean litter weights and pup mean body weights were significantly lower than in controls by Day 25 post-partum. In the F1->F2 litters, group mean litter weights were slightly lower throughout lactation. Litter size was slightly lower than in controls from birth to weaning in the F1->F2 litters.

At 600 ppm, group mean pup body weight gain was slightly reduced (by about 9%) by Day 25p.p. in the F0->F1 litters alone although group mean litter weights tended to be marginally lower than controls in both littermgs.

The only effect on reproduction was a marginal lowering of litter size in litters from the F1 generation at 6000 ppm, the highest dose level.

On the basis of results obtained no effect levels were judged to be;
- **Toxicity**: 600 ppm (equivalent to 4.9 mg/ kg/day)
- **Reproduction**: 60 ppm (equivalent to 50 mg/kg/day)

(Schering Agrochemicals Ltd., 1992)

2. **Rat Teratology Study**

Dose levels of 0 (control), 3, 55 or 1000 mg/kg, in 1 % w/v methyl-cellulose in distilled water, were administered once per day by gavage to groups of 25 time-mated CD(SD) strain females from Day 6 to Day 15 post coitum inclusive. On Day 20 the animals were killed, litter values were determined and foetuses subsequently examined for visceral and skeletal changes. The following findings were observed.

**Maternal findings**: Treatment with technical benfuresate was associated with a dose-related increase in transient post dosing salivation. At 1000 mg/kg/day, this sign occurred in all females, generally throughout the treatment period, and was associated with wet coats and, occasionally, brown staining of the coat. Smaller numbers of animals at lower dose levels exhibited salivation for shorter time periods.

At 1000 mg/kg/day, there was a progressive and significant increase in water consumption throughout the treatment period. Differences from control in water consumption persisted to termination but decreased after the end of treatment.

There were no other conclusive treatment-related responses at lower dose levels.

**Litter findings**: Litter parameters appeared unaffected in all treatment groups, as judged by embryofoetal survival, foetal growth and morphological development.

On the basis of results obtained, the no effect level (NOEL) for maternal parameters was 3 mg/kg/day and for embryofoetal development was 1000 mg/kg/day.

(Huntingdon Research Centre, 1992)

3. **Rabbit Teratological Study**
Dose levels of 0 (control), 50, 200 or 800 mg/kg, in 1 % carboxymethylcellulose in distilled water, were administered once per day by gavage to groups of 16 mated females of Chinchilla rabbits from Day 6 to Day 18 post coitum inclusive. On Day 28 post coitum, the dams were killed, litter values were determined and foetuses subsequently examined for visceral and skeletal changes. The following findings were observed.

Maternal findings: Two females at 200 mg/kg died during the dosing period, being attributed to intubation error. There were no mortalities, behavioural changes or necropsy findings in the mated females considered to be related to treatment with test article.

At 800 mg/kg, weight gain was slightly retarded during the first five days of dosing and food consumption was reduced during the first nine days of dosing and during the last four days of the study. At 200 and 50 mg/kg, weight gains and food consumption were similar to those of controls throughout the study. There were no treatment-related differences in the values for water consumption, liver and kidney weights and the reproduction parameters-overall pregnancy rate, incidence of total resorption, numbers of implantations, pre- and post-implantation losses and numbers of live fetuses.

Litter findings: Litter parameters were not affected by treatment, as judged by foetal growth and morphological development.

On the basis of results obtained, the no effect level (NOEL) for maternal parameters was 200 mg/kg/day and there was no evidence of an embryotoxic or teratogenic effect up to the highest dosage examined, 800 mg/kg/day.

(Research & Consulting Company, 1988)

MUTAGENICITY STUDIES

1. Bacterial Reverse Mutation Assay
The potential of benfuresate to induce reverse gene mutations was evaluated in Ames plate incorporation assays employing the histidine auxotrophic Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 in both presence and absence of an Aroclor 1254-induced rat liver metabolic activation system (S-9). A top dose level of 5000 mg/plate was chosen and other dose levels used were 1500, 500, 150 and 50 mg/plate. The test system was validated with concurrent positive control using 9-aminoacridine (9-AC), N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG), 2-nitrofluorene and 2-aminoanthracen (2-AA). A number of revertant colonies did not increase when cultured with or without metabolic activation system at any concentration of test article, indicating benfuresate did not induce gene mutation.

The potential of benfuresate to induce reverse gene mutations was also evaluated in the tryptophane auxotrophic Escherichia coli strain WP2 uvrA in both the presence and absence of rat liver metabolic activation system. The same dose levels as those in
the study with *S. typhimurium* was used, and the test system was validated with concurrent positive control using ENNG and 2-AA. No mutagenic effect was observed. (Huntingdon Research Centre, 1991)

2. *Mammalian Cytogenetics*

The potential of benfuresate to induce chromosomal aberrations in human lymphocytes cultured *in vitro*.

Concentrations of technical benfuresate dissolved in ethanol, used for metaphase analysis were 15, 75 and 150 μg/ml in the absence of S-9 and 75, 375 and 750 μg/ml in the presence of S-9. No significant increase in the number of aberrant cells was observed in treated groups compared with the solvent control. Both positive control compounds, ethylmethane sulphonate for the absence of S-9 and cyclophosphamide for the presence of S-9 caused statistically significant increases in the proportion of metaphase spread containing aberration. It is concluded that when tested up to the limits of solubility in ethanol, benfuresate showed no evidence of clastogenic activity and therefore no evidence of mutagenic activity in this *in vitro* cytogenetic assay. (Huntingdon Research Centre, 1984)

3. *Bacterial DNA Repair Test*

The potential of benfuresate to damage DNA was evaluated *in vitro* in a bacterial recombination assay using *Bacillus subtilis* strains H17 (rec*) and H45 (rec⁻) both in the presence and absence of rat liver metabolic activation system. A top dose level of 5000 μg/plate, and other dose levels, 1500, 500, 150 and 50 μg/plate were used. Kanamycin and streptomycin as negative controls, AF-2, and 2-AA as positive controls were used to confirm the sensitivity, efficacy and integrity of this test system. No genetic damage was observed. (Huntingdon Research Centre, 1991)

**GENERAL PHARMACOLOGY**

1. *Effect of General Behaviour of Mice*

Benfuresate was assessed for the behavioural and autonomic effects following a single oral dose of 62.5, 125, 250, 500, 1000 or 2000 mg/kg to 5 male ICR mice per dose level using modified Irwin dose-range test. Irregular and slow breathing, transitory increase of motor activity and slight depression of motor activity were seen in all treated groups, and findings of midriasis were observed in animals administered 250 mg/kg. Tremors in 1000, 2000 mg/kg, ventricumbent, no gripping strength and sialorrhoea in 2000 mg/kg were seen. One animal in 2000 mg/kg group died by 1 day after dosing, and the additional findings, gait spastic, spasmodic twitches, abolished pina and light-pupil reflex, prolonged response time and marked hypothermia were observed in this animal.

Findings of hypothermia were seen in animals treated with 500, 1000 and 2000
mg/kg. The peak of occurrence of these findings was about 1 to 3 hr after dosing, and the findings recovered completely within 1 day after dosing.

2. Effects on Respiratory and Circulatory Systems
The effects on respiratory and circulatory parameters in 5 anaesthetised male rabbits administered intravenously at doses of 1 and 10 mg/kg were examined.

Benfuresate at a dose of 10 mg/kg produced a significant decrease of mean blood pressure and a significant increase of respiration rate which was observed from immediate after dosing to 5 min comparing to solvent control.

In a group at a dose of 1 mg/kg, no specific finding was recorded.

3. Effects on Spontaneous Motility of Isolated Ileum
Antagonist action of benfuresate against acetylcholine, histamine and barium chloride was assessed at concentrations of $3.0 \times 10^{-8}$ to $3.0 \times 10^{-5}$ g/ml with the ileum isolated from Guinea pigs. Significant inhibitions were observed on the contraction induced with three antagonists at the concentration of $10^{-5}$ g/ml, comparing to solvent control.

4. Effects on Gastro-intestinal Propulsion
Activated charcoal suspension was given orally to mice 60 min after oral administration of benfuresate at 250, 500 and 1000 mg/kg. Twenty minutes after administration of the suspension, the animals were sacrificed and propulsion of the charcoal to intestine determined.

No effect of benfuresate was observed in all treated groups, while atropine sulfate at 20 mg/kg used as a positive control showed significant inhibition of propulsion of activated charcoal.

5. Effects on Neurowascular Junction
The effect of benfuresate on neuromuscular junction was assessed using phrenic nerve diaphragm specimens from male rats. The change in contraction of isolated rat diaphragm muscle by electrical stimulation of phrenic nerve and muscle was measured following treatment with benfuresate at concentrations of $3 \times 10^{-7}$ to $3 \times 10^{-5}$ g/ml.

Compared to ethanol treated animals, benfuresate at a concentration of $3 \times 10^{-5}$ g/ml produced significant increase of contraction indirectly by stimulation of nerve. No effect was observed for contraction of diaphragm muscle by direct stimulation.

6. Effect on Blood Coagulation System
The effects of benfuresate on blood coagulation system were assessed in 5 male rats administered by gavage at a dose of 1000 and 2000 mg/kg.

Benfuresate did not produce any effects on thrombin, prothrombin or partially activated thromboplastin time either in 1000 mg/kg or in the 2000 mg/kg.
7. Effect on Haemolysis in vitro

The potential of benfuresate to cause haemolysis was assessed by mixing 0.02 and 0.2 mg/ml benfuresate with blood of rabbit, and measuring the optical density at 541 nm. No significant differences from the 1% ethanol/saline group observed in both treated group suggesting that benfuresate did not cause haemolysis.

(Nihon Schering K.K., 1992)

SUMMARY

Technical benfuresate was of very low acute oral and dermal and low acute inhalational toxicity to rodents. Repeat dose studies in the rat, mouse and dog showed the material to be well tolerated with the maximally tolerated doses of 318, 561 and 400 mg/kg/day, respectively. Histopathology showed the kidney to be the main target organ in all three species. The principal findings in the kidney, found following subchronic exposure in the rat and following subchronic/chronic exposure in the mouse and dog were papillary necrosis and dilatation and/or degeneration of the proximal tubules (and collecting ducts in 'rats). These findings were associated with elevated kidney weights in the rat and dog, pyelonephritis in the mouse and dog and interstitial nephritis in the rat and dog.

The liver was also a target organ at dose levels above the MTD in the rat and mouse as indicated by swelling of the periportal hepatocytes and centrilobular enlargement, respectively.

There was no evidence of oncogenicity in the mouse or rat and the NOEL in the most sensitive species, the rat, was 3 mg/kg/day. Technical benfuresate was non-genotoxic, non-irritant, had no effect on reproduction or fertility and was not a skin sensitiser.

The compound was rapidly and completely absorbed. Tissue residues were low and declined rapidly. The highest levels were found in the kidney and fat after 6 hr. Metabolism of the compound in the rat was rapid and complete as was elimination, which was predominantly via the urine.

The compound showed weak pharmacological effects on the nervous system following oral administration and the circulatory and respiratory systems, but only after intravenous dosage.

The acute toxicity of metabolites, process intermediates and formulations of benfuresate were low.

It is concluded therefore that under the recommended conditions of use both technical benfuresate and formulations are unlikely to present hazard to operators or the public who may consume negligible residues from treated produce.

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