

(b) After 4.8 hours of direct intraurethral instillation, about 25% of  $C^{14}$  had disappeared from the bladder contents and was retained by several body tissues or  $CO_2$  - Searle Project P-T 1038 of 72 (Appendix 37A1).

## V. Mutagenicity studies

### In vivo cytogenetics

- Species and strain: Rat - Purina Caesarian-derived (8 weeks old)
- Group size : 10 ♂
- Route : Intragastric administration of negative control and DKP in 3 equally divided doses every 3 hours for 5 days and intraperitoneal administration of positive control as a single dose on day 5 only.
- Dose levels : Negative control - 40 ml/kg/day 1% aqueous Tween 80  
DKP - 0.25, 0.5, 1 or 2 g/kg/day  
Positive control - 0.5 mg/kg triethylenemelamine (TEM)
- Duration of treatment: 5 days (see route above). 24 hours after day 5 of treatment, all rats were injected ip with 4 mg/kg colcemid and killed 5 hours later.

### RESULTS

- Appearance and behaviour : Death occurred on day 3 in one rat given 1g/kg DKP; cause of death was not determined at necropsy. A slight increase in the incidence and frequency of soft stools was observed in the group given 2g/kg/day DKP when compared with other groups.
- Growth : No treatment-related effects when compared with negative controls; all groups, with the exception of the positive controls, lost weight, though rats given DKP lost slightly more than negative controls.
- Food consumption : Food consumption was comparable between negative controls and DKP groups, however, it was slightly higher in the positive control group.
- Bone marrow cytology : No effects attributable to treatment with DKP were observed. Positive control group exhibited a significantly greater incidence of cells with aberrations.
- No-untoward-effect level : 2g/kg
- Reference : Searle Project No P-T 1027 H72 (Appendix 33)

### Host-mediated assays

(i) Species and strain: Mouse - Swiss Ha/ICR (5-6 weeks old)

Group size : 10 ♂

Route : Oral administration of negative control and DKP in 3 equally divided doses at 2-hour intervals for 5 days and of positive control as a single dose on day 5 only.

Dose levels : Negative control - 40 ml/kg/day of 1% aqueous Tween 80  
DKP - 1, 2, 4 or 8 g/kg/day  
Positive control - 0.020 g/kg methylazoxymethanol.

Duration of treatment: 5 days; 30 minutes after the last dose, all mice were injected ip with Salmonella typhimurium strain G46 (a histidine auxotroph). The mice were killed 3 hours later and all peritoneal fluid removed for culturing.

### RESULTS

Mortality : 100% survival was achieved in both control groups and the lowest DKP group; however in the medium, high and very high dose groups survival rates were 90, 80 and 30% respectively. Although cause of death was not determined, all mice had severe diarrhoea and subsequent dehydration. The deaths occurred between days 3 and 5 of treatment. In addition, 4 mice (one negative control, two low dose DKP and one high dose DKP) died following the ip injection of bacteria.

Growth : On day 5 weight loss of negative control and DKP groups was similar (no figures given during treatment period). The positive control group gained weight slightly.

Food consumption : No treatment-related changes; the positive control group ate a significantly greater amount of food.

Mutation frequency: No treatment- or dose-related changes were observed. A significantly higher mutation frequency was observed in the positive control group.

No-untoward-effect level : 8 g/kg (for mutation frequency only).

Reference : Searle Project No 1095 S73 (Appendix 35A).

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(ii) Species and strain: Rat - Purina Caesarian-derived (12 weeks old)

Group size : 10 ♂

Route : Oral administration of negative control and DKP in 3 equally divided doses daily for 5 days and intra-peritoneal administration of positive control as a single dose on day 5 only.

Dose levels : Negative control - 40 ml/kg/day of 1% aqueous Tween 80  
DKP - 0.25, 0.5, 1.0 or 2.0 g/kg/day  
Positive control - 100 mg/kg dimethylnitrosamine.

Duration of treatment: 5 days; 30 minutes after the last dose, all rats were injected ip with Salmonella typhimurium strain G-46 (a histidine auxotroph). The rats were killed 3 hours later and the peritoneal fluid removed for culturing.

## RESULTS

Appearance and behaviour : The incidence and frequency of soft faeces was increased in a dose-related manner in all DKP groups. Two rats given 2g/kg/day died one each on days 3 and 5; cause of death was not determined.

Growth : All negative control and DKP rats lost weight, with a slightly greater, but not dose-related, loss occurring in DKP groups. Positive control increased in weight.

Food consumption : Similar consumption in negative control and DKP groups and greater consumption in positive control group.

Mutation frequency: No treatment- or dose-related changes were observed; the frequency of mutations in the positive control group was significantly increased.

No-untoward-effect level : 2g/kg

Reference : Searle Project No P-T 1029 H72 (Appendix 34).

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## Dominant lethal assay

Species and strain: Rat - Charles River CD

Age at start of study : Proven fertile males - 160 days  
Untreated virgin females - 90-130 days.

Group size : 15 ♂ (negative control and DKP), 10 ♂ (positive control)

Route and dose level : Males only: intragastric administration of 1% aqueous Tween 80 (negative controls) or 1 g/kg DKP, both given as 2 equally divided doses 2 hours apart on day 1 only; intraperitoneal administration of a single dose of 50 mg/kg methyl methane sulphonate (MMS) (positive controls on day 1 only.

Mating procedure : Immediately following treatment, each male was paired with two females for a period of one week, after which time two new females were presented to the male. This procedure lasted 8 weeks. The presence of a copulatory plug and/or spermatozoa in the vaginal smear was considered as evidence of mating and hence day "0" of gestation. Females unmated after one week's exposure to a male were removed, retained for 12 days, then killed and autopsied.

Length of pregnancy : 14 days

## RESULTS

Males : Appearance and behaviour : Observed daily; no treatment-related changes were observed.

Growth : Bodyweights recorded weekly; rate of bodyweight gain was similar in all groups.

Food consumption : Not recorded.

Females: Growth and food consumption : Not recorded.

Pregnancy effects : Once killed, the uterine horns were examined for implantations (classified as viable fetal swellings or early or late deaths) and the number of corpora lutea were recorded. Overall pregnancy rates were similar between negative control and DKP groups and significantly reduced in the positive control group. No treatment-related changes were observed in any of the parameters monitored; however, the positive control group exhibited an increase in mean number of fetal deaths and a decrease in mean number of viable fetal swellings during weeks 1-5. A reduced implantation rate was observed in this group during weeks 2-5 but this was partially due to an unexplained decrease in ovulation rate in weeks 2-4.

Post-mortem observations: Gross observations were made of the lungs, uterus and ovaries. A raised, nodular appearing pleural surface and pleural adhesions extending to the thoracic wall and/or diaphragm were observed in 2/211 "negative controls", 11/240 "DKP females and 0/160 "positive control" females.

No-untoward-effect level : 1g/kg

Reference : Searle Project No 1008 S72 (Appendix 35).

## VI. Reproduction studies

Species and strain: Rat - Charles River CD albino

Age : 90 days at start of treatment; animals bred when males approximately 140 days and females 105 days of age.

Group size : 12 ♂ + 60♀ (controls); 12 ♂ + 28♀ (DKP)

Route and dose levels : Dietary administration of 0, 0.5, 1.0 or 2.0 g/kg/day (Groups C, L, M and H respectively); during mating compound was administered intragastrically, with controls receiving 1% aqueous Tween 80 only.

Duration of treatment : ♂ until the end of the mating period.  
♀ until the end of the lactation period.

Mating procedure : The rats were randomly subdivided into 48 mating units, each containing 1 male and 3 females, to produce the following litter combinations:-

No of pregnancies produced ( $\sigma^7 \times \text{f}$ )

CC	LL	MM	HH	CL	CM	CH	LC	MC	HC
24	24	24	24	4	4	4	12	12	12

Rats were allowed to mate overnight for up to 4 weeks and evidence of copulation was ascertained by the presence of a vaginal plug and/or spermatozoa in the vaginal smear. Half of the pregnant rats were killed on day 14 of gestation and the remainder allowed to give birth and raise the young to weaning.

RESULTS

- Males : Appearance and behaviour : Examined weekly during the premating period and daily during mating; no adverse effects were recorded and survival was unaffected by treatment with DKP.
- Growth : Bodyweights were recorded at weekly intervals; no treatment-related effects noted.
- Food consumption : Measured biweekly during the premating period only. During weeks 1-4 of the premating period, consumption of all DKP groups exceeded that of the controls in a dose-related manner; however during weeks 5-7 of premating food consumption was similar in all groups.
- Females: Appearance and behaviour : Examined weekly during the premating period and periodically during pregnancy and lactation. No treatment-related effects, survival was 100% in all groups.
- Growth : Bodyweights recorded at same intervals as for appearance and behaviour. No treatment-related changes.
- Food consumption : Measured biweekly during premating and daily during pregnancy and lactation. No treatment-related effects. Intake of DKP was similar to the planned levels of 0.5, 1 or 2 g/kg but during the final week of lactation DKP intake rose to 0.66, 1.05 and 2.12 g/kg for the respective groups.
- Hysterotomy data : Of dams killed on day 14, the ovaries and uterus were examined for gross changes, the number and position of live dead and resorbed fetuses counted and the numbers of corpora lutea recorded; no adverse effects were noted.
- Conception rate : Unaffected by treatment.
- Litter data : On days 0, 4 and 21 all pups were fully examined, sexed and weighed. No adverse effects were noted in number of viable pups, sex ratio, and bodyweights.
- Pup survival : Unaffected by treatment (days 4 and 21).

Pup gross examination : Unaffected by treatment (days 0, 4 and 21)

Ophthalmoscopy : All weaned pups of the high dose group only examined between days 21 and 30. No effects observed at a frequency that could be attributable to treatment with DKP.

No-untoward-effect level : 2g/kg

Reference : Searle Project No 996 S72 (Appendix 46).

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#### Teratology studies

(i) Species and strain : Rat - Charles River CD albino.

Group size : 28<sup>♀</sup> (+ 7 <sup>♂</sup> proven fertile)

Route and dose levels : Dietary administration of 0, 0.5, 1 or 2 g/kg/day DKP.

Duration of treatment : Days 6-15 of pregnancy. (Females only)

Mating procedure : Rats were allowed to mate overnight, one male to four females, copulation being ascertained by the presence of a vaginal plug and/or spermatozoa in the vaginal smear. 24 "mated" females were selected per group for the feeding study. Dams were killed on day 20 of pregnancy by CO<sub>2</sub> asphyxiation.

#### RESULTS

Appearance and behaviour : Monitored daily; no adverse treatment-related effects.

Growth : Dams weighed periodically throughout the study; no adverse effects, though in high dose dams, only, rate of bodyweight gain was slightly greater than that of controls towards the end of gestation.

Food consumption : Recorded daily; no treatment-related effects.

Hysterotomy data : Uterus and ovaries were examined grossly; numbers of implantation and resorption sites, viable and non-viable pups recorded and pups examined for weight, sex, crown-rump length, and gross, visceral and skeletal abnormalities. Conception rate was slightly reduced at the highest dose level, though not significantly. No adverse effects were noted in any of the parameters.

No-untoward-effect level : 2 g/kg

Reference : Searle Project No 997 S72 (Appendix 47).

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(ii) A second teratology study in the rat was performed feeding dams a 3:1 mixture of aspartame: DKP on days 6-15 gestation. The results of this study (Searle Project No PT-1001 H72 (Appendix 40)) have been reported in Appendix I, Section VII.

(iii) Species and Strain: Rabbit - New Zealand White

Group size : 21-22 ♀ (artificially inseminated).

Route : Intragastric administration of vehicle control or DKP as two equally divided doses, 4 hours apart.

Dose levels : 0, 0.5, 1.0 or 2.0 g/kg/day

Duration of treatment : Days 6-18 of gestation. Does killed on either days 28 or 29 of gestation.

#### RESULTS

Does: Appearance and behaviour : Monitored daily; of the does which survived the study, no treatment-related effects were observed. However, mortality rate was high viz 6/21 controls, and 4/21, 6/22 and 19/21 of the low, medium and high dose groups respectively. All animals were autopsied and in most cases of the high dose does, it was thought that death was secondary to a physical blockage of the pyloric sphincter caused by massive accumulations of substance (thought to be DKP) admixed with hair. In addition most cases of death were preceded by anorexia, weight loss and soft faeces.

Growth : Bodyweights recorded on days 0, 6, 10, 15, 18, 22 and 28/29 of gestation (Data analysed for surviving pregnant rabbits only). Rate of bodyweight gain was unaffected by treatment at the two lower dose levels; however the one surviving pregnant rabbit given 2 g/kg/day lost more weight than controls during the first 5 days of DKP treatment and also post-treatment.

Food consumption : Recorded daily; consumption unaffected by treatment of up to 1 g/kg/day DKP. During the period of DKP administration a reduced food intake was observed in the one survivor given 2 g/kg/day.

Terminal studies : Numbers of corpora lutea and implantation and resorption sites were recorded and all does found dead or killed at term were subjected to a gross autopsy to include the uterine and visceral structures. No adverse treatment-related effects were observed.

Litter examination: Numbers of live and dead fetuses were recorded and all fetuses were examined for sex, weight, crown to rump length and gross, visceral and skeletal abnormalities. No treatment-related effects noted.

No-untoward-effect: level : 1 g/kg (At the higher level, there were insufficient animals for statistical analysis.)

Reference : Searle Project No PT 1003 H72 (Appendix 57).

- (iv) A second teratology study in the rabbit was performed feeding the does a 3:1 mixture of aspartame: DKP on days 6-18 of gestation. The results of this study (Searle Project No PT 1002 H72 (Appendix 51)) have been summarised in Appendix I section VII of this paper.

Late fetal and early post-natal study

Species and strain: Rat - Charles River CD albino.

Group size : 20 mated ♀ (dams mated with proven fertile males).

Route and dose levels : Dietary administration of 0, 0.5, 1 or 2 g/kg/day DKP (0, 0.66, 1.32 and 2.63% respectively of the diet).

Duration of treatment: From day 14 of gestation until pups weaned.

RESULTS

Dams: Appearance and behaviour : Monitored daily; no treatment-related effects; all groups achieved 100% survival rate.

Growth : Bodyweights recorded weekly during gestation and lactation. Rate of bodyweight gain was similar in all groups with no dose-related trends.

Food consumption : Recorded weekly; no adverse effects. Intake of DKP during gestation was within 10% of the planned levels; however by the end of lactation DKP intake had risen to 0.67, 1.31 and 2.54 g/kg/day in the low, medium and high dose groups respectively.

Litter examinations : Within 24 hours of birth all litters were reduced to 8 pups. On days 0, 4 and 21 post-partum the following observations were made:- number of viable and non-viable pups, gross external examinations of all pups, sex, weight and non-viable pups were examined for visceral and skeletal abnormalities. No adverse effects were noted in any of the parameters.

Pup Ophthalmoscopy: Pups from the high dose group were examined between days 40 and 42 of age and from the control group when aged 47-59 days. No adverse effects were noted that could be related to treatment.

No-untoward-effect level : 2 g/kg

Reference : Searle Project No PT 1011 H72 (Appendix 48).

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SUMMARY OF BIOLOGICAL STUDIES ON ASPARTAME

Study	Animal Species	No of Studies	No-effect level (NEL)	Comments
Sub-acute	Mouse Rat	1 1	13g/kg 10g/kg	
Short-Term	Rat Dog  Monkey	2 1  1	2.5g/kg 125mg/kg  1g/kg	In one study raw data <u>unavailable</u> . Highest dose administered was 125mg/kg/day. Insufficient number of organs available at autopsy to detect changes in liver and kidney.  No conventional controls used. Grand mal convulsions observed in groups given 3 and 6 g/kg/day.
Long-Term (carcinogenicity)	Hamster  Rat  Mouse  Dog	1  2  1  1	8.3g/kg  2g/kg  2g/kg  2g/kg	Study terminated due to disease in colony (wet tail) after 46 weeks.  One study incorporated a period of <u>in utero</u> exposure. Decreased survival at 8g/kg/day. Bodyweight gain and food consumption depressed at 4g/kg/day.  Increase in absolute and relative heart weights at 4g/kg/day, though no histological changes observed.  Decrease in haemoglobin, haematocrit and erythrocytes in males given 4g/kg/day throughout much of the study.
Bladder tumourigenicity (26 and 56 weeks)	Mouse	1	Implantation of 4g	



SUMMARY OF BIOLOGICAL STUDIES ON DKP

Study	Animal Species	No of Studies	No-effect level (NEL)	Comments
Sub-acute (2 weeks) (5 weeks)	Mouse Rat Rat	1 1 1	1g/kg 1g/kg 4g/kg	At 6g/kg observed decreased weight gain, significant reductions in absolute weights (in particular females) of adrenals, ovaries, pituitary and uterus.
Long-Term	Mouse Rat	1 1	1g/kg Not determined	Uterine changes - (abcessation, endometrial hyperplasia and polyps) observed in dose-related manner. Independent assessment resulted in opinion that changes were benign and part of the aging process in the rat.
Bladder tumourigenicity (26 + 56 weeks)	Mouse	1	4g im-plant	Incidence of bladder neoplasia after 26 weeks higher than positive controls, but comparable with negative controls after 56 weeks.
Mutagenicity	Rat Mouse Rat Rat	1 1 1 1	2g/kg 8g/kg 2g/kg 1g/kg	<u>in vivo</u> cytogenetics Host-mediated assay " Dominant lethal assay
Reproductive performance	Rat	1	2g/kg	Males dosed 50 days and females 15 days prior to mating; females dosed until end of lactation.
Teratology	Rat Rabbit	1 1	2g/kg 1g/kg	Mortality at 2g/kg was 19/21, hence insufficient animals (only 1 was pregnant) for statistical analysis at this level.
Late fetal/early post-natal	Rat	1	2g/kg	

# HUMAN STUDIES

Duration of treatment	Age of subject	NEL
6 weeks	Normal adults Obese adults PKU heterozygous adults	) 0.6 (week 1) - 8.1 (week 6) ) g/day )
21 weeks	Normal adults Obese adults PKU heterozygous adults  Normal children	) ) 1.8g/day )  40-58mg/kg/day
13 weeks	Diabetics	1.8g/day
Loading doses (single dose)	Normal adolescents PKU hom ozygous children Lactating females	34mg/kg 34mg/kg 50mg/kg