



The second study is currently ongoing and is sponsored by the producers of phthalate esters under two umbrella organizations, the European Council of Plasticizers and Intermediates and the Chemical Manufacturers Association. The study is being conducted at TNO laboratories in the Netherlands. A protocol for this study is enclosed as Attachment II. This study is a replicate of the original Sharpe protocol with the following enhancements added to that protocol: (1) instead of only one dose level of 1ppm in the drinking water, 3 dose levels (0.1, 1.0 and 2.5 ppm and the high dose at the limit of solubility of BBP in drinking water) were used in the present study (2) a much higher number of test animals per group were used (3) the dosing solutions were analytically confirmed throughout the course of the study (4) glass bottles were used for administering BBP to limit any potential contamination from plastic bottles (5) IVOS technique was used instead of manual counts for daily sperm production counts. The revised technique was validated in consultation with Dr. Sharpe.

We obtained preliminary data from the laboratory which show that BBP at the dose levels tested did not produce any statistically significant changes in either testicular weights or the daily sperm production counts (Attachment III, Tables 1 and 2). Thus, like Ashby et al., we were unable to duplicate Dr. Sharpe's findings on the effects of BBP on testicular weights and daily sperm production counts. We also understand that at the recent EDSTAC Workshop on low dose effects John Ashby revealed that Dr. Sharpe will be sending a letter to Environmental Health Perspectives withdrawing his study.

In our study, preliminary parturition data showed an anomalous increase in the number of stillborn pups and postnatal mortality (lactation days 1-4) in the 1 and 2.5 ppm dose groups on a pup basis but not on a litter basis (Attachment III, Table 3). As discussed below, the effects observed are not considered to be treatment related. Note that neither Sharpe nor Ashby observed this effect in studies of the same design, and these effects have not been observed in a conventional one-generation (2 litters) with BBP in the diet at significantly higher concentrations (2000, 4000 and 8000 ppm). A comparison of the concurrent control data with the historical control data for this parameter strongly suggests that the concurrent control values exceed the range of the historical controls at the laboratory. The results were also confounded by a short power outage during the lactation period.

An additional mating was performed with 3 extra groups of 28 females each and the study was terminated at lactation day 7. Note the dosing regimen for the additional experiment was identical to the first mating. The results of the additional mating (Attachment III, Table 4) are themselves inconsistent with the previous mating in that the incidence of stillborn pups and postnatal mortality in the concurrent control group (14%) is almost twice as high as what was observed in the first mating. The incidence in the 1.0 ppm BBP dose group (7.6%) is significantly less than the concurrent group. The incidence in the 2.5 ppm BBP remains higher than concurrent controls on a pup basis. The laboratory has informed us that there has been a significant shift in the incidence of stillborn and postnatal deaths (lactation day 1-4) in its contemporaneous experience since

two other reproduction studies performed with different compounds during the same time period showed similar increases in either the control and/or the treated animals. A copy of the letter from the laboratory is attached (Attachment IV). This confounding factor, the inconsistency of response in the current study, and the inconsistency of the observation with other studies both with similar design and at much higher concentrations indicate that the observations are not related to the administration of BBP. We are providing this update to you as we have communicated with the Agency that this study is ongoing. A final report is expected to be available in May 1998, and a copy will be provided to you at that time.

We also want to make the Agency aware that the other producers of BBP, Bayer AG and Lonza S.P.A. are aware of our submission.

Sincerely,

A handwritten signature in black ink, appearing to read "Jeffrey D. Felder". The signature is fluid and cursive, with a prominent loop at the end.

Jeffrey D. Felder, P.E.  
Leader, Product Stewardship

Attachments: 4  
as

# Monsanto

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December 12, 1995

Document Processing Center (TS 790)  
Office of Pollution Prevention and Toxic Substances  
United States Environmental Protection Agency  
401 M Street, SW  
Washington, DC 20480

## ATTENTION 8(e) COORDINATOR

Dear Sir or Madam:

The information in this letter pertains to butyl benzyl phthalate and is being submitted to the U.S. Environmental Protection Agency in accordance with the EPA's interpretation of Section 8(e) of the Toxic Substances Control Act. Although we do not believe that this information constitutes a substantial risk for all of the reasons discussed below, the present submission is intended to discharge any 8(e) responsibilities that might exist and thus should be processed in accordance with the EPA's "substantial risk" procedures.

With this letter Monsanto submits to the TSCA 8(e) file a preprint to be published in *Environmental Health Perspectives* entitled "Gestational/neonatal exposure of rats to environmental estrogenic chemicals results in reduced testicular size and sperm production in adult life" by Sharpe, Fisher, Millar, Jobling and Sumpter. In this study the authors purport to show that treatment with butyl benzyl phthalate (and several other chemicals) during pre- and neonatal development results in reduced testicular size and in a concomitant reduction in sperm count in adult rats.

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We do not believe that these data represent a substantial risk because of limitations in the experimental design (the choice of negative control was inappropriate and only one dose level of butyl benzyl phthalate was tested) and because of the small magnitude of the changes reported with butyl benzyl phthalate (6.6-7.5% reductions in relative testicular wt. with concomitant reductions in sperm count per teste). Further, these findings appear inconsistent with results from other toxicity studies conducted with BBP under a number of different study, route and dosing conditions.

We also take this opportunity to note that Union Carbide has submitted to this office a copy of this same preprint because it contained data pertaining to 4-tert-octylphenol which were broadly similar to that described above for butyl benzyl pthalate.

Sincerely,



Roger M. Weppelman, Ph.D.  
Manager, Regulatory Affairs.

TNO protocol  
P470839

**Protocol for an oral developmental reproduction study with  
butyl benzyl phthalate in Wistar rats**

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Submitting the protocol for inspection to  
parties who have a direct interest is  
permitted.

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1

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## 1 Objective of the study

The purpose of this study is to investigate the reproducibility of, and expand on, the findings of Sharpe et al. (Environmental Health 103: 113, 1995) related to the development of the reproductive system in Wistar rats exposed *in utero* and during lactation to butyl benzyl phthalate in drinking water.

The study will be conducted according to the OECD Principles of Good Laboratory Practice.

## 2 Sponsor

European Council for Plasticizers and Intermediates

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## 3 Testing facility

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## 4 Responsible personnel

Study director	: Ir D.H. Waalkens-Berendsen <sup>1</sup>
Assistant study director	: A. Dijkstra
Deputy study director	: Ir D. Jonker
Responsible for animal care	: G. van Beek
Responsible for pathology	: Dr R.A. Woutersen
Responsible for test substances	: E.A. de Vries
Responsible for analyses	: Dr E.R. Verheij (Principal Investigator)

<sup>1</sup> Toxicology Division, TNO Nutrition and Food Research Institute

## 5 Time schedule

a. Planned arrival of the animals	: 29 April 1997
b. Planned experimental start date	: 12 May 1997
c. Planned experimental termination date	: September 1997
d. Planned reporting date (draft report)	: within 3 months after termination of the in-life part

## 6 Test substance

### 6.1 Characterization of the test substance

The test substance, butyl benzyl phthalate is supplied by Monsanto Europe S.A., Brussels, Belgium.

Chemical name	: butyl benzyl phthalate (BBP)
CAS. reg. no.	: 85-86-7
Batch number	: LLN 96046 (6B1008)
Purity	: 99.1 % /w
Appearance	: clear oily liquid
Storage conditions	: at ambient temperature

Isobutyl benzyl phthalate, internal standard is supplied by Monsanto Europe S.A., Brussels, Belgium.

Chemical name	: isobutyl benzyl phthalate (iBBP)
Batch number	: LLN 95094 (5569117)
Purity	: 98.7 % /w
Appearance	: white crystals
Storage conditions	: at ambient temperature

The positive control substance, diethylstilbestrol is obtained from Aldrich Chemie, Zwijndrecht, The Netherlands.

Chemical name : diethylstilbestrol (DES)  
CAS. reg. no. : 56-53-1  
Batch number : 64H0690  
Purity : 99%  
Appearance : white, crystalline  
Storage conditions : in the dark at ambient temperature

## **6.2 Non-routine health and safety requirements**

To prevent exposure to DES, the following specific precautions should be taken when handling the animals, drinking water solutions, cages, etc.: protective gloves and suit, and eye protection. Waste (remaining drinking water solutions containing DES, DES stock solutions, urine and faeces, carcasses) will be collected for incineration.

## **7 Test system**

### **7.1 Characterization of the test system**

The study will be conducted with albino rats. The rat is used because this species is considered one of the most suitable species for this type of study and this species was used in the study by Sharpe et al. 1995.

About 74 male and 148 female rats, Wistar outbred (CrI:(WI)WU BR) of about 9-10 weeks old will be ordered from a colony maintained under SPF-conditions at Charles River Wiga GmbH, Sulzfeld, Germany.

### **7.2 Animal allocation**

Upon arrival, the rats will be taken to a quarantine room and checked for overt signs of ill health and anomalies. During the quarantine period serological investigation of the microbiological status will be conducted in random samples. If the results of serology are satisfactory, the rats will be transferred to their experimental room. Subsequently they will be acclimatized to the laboratory conditions for at least 5 days prior to the experimental start date. Shortly before the start of the study, the animals will be allocated to the various groups by computer randomization on the basis of body weight.

Surplus animals will be kept in the animal room for monitoring during the study. They may be used in the study proper when necessary, otherwise they will be discarded at the end of the study.

### **7.3 Identification of the test system**

During the acclimatization period, the animals will be identified by a temporary tailmark.

#### **F0 (parent)-animals:**

Following allocation, shortly before the start of the study, the individual female rats will be identified by a unique animal identification number which will be tattooed and clipped in the ears. Males will be identified by the animal identification number on their cage card.

#### **F1-animals:**

Pups will be identified individually within the litter on postnatal (PN) day 21. Before PN day 28 they will be marked with a unique animal identification number which will be tattooed and clipped in the ears.

Males will be identified by even numbers and females by odd numbers. Each dosing group will be coded by a letter and a colour (see section 9.3). Each cage will be provided with a card showing the colour code, animal identification number(s), cage number, group letter and study number.

## **8 Experimental conditions**

### **8.1 Animal maintenance**

The animals will be housed under conventional conditions in one room reserved for this study. No other test system will be housed in the same room during the study. The room will be ventilated with about 10 air changes per hour and will be maintained at a temperature of 22 +/- 3°C and a relative humidity of at least 30% and not exceeding 70% other than during room cleaning. Lighting will be artificial with a sequence of 12 hours light and 12 hours dark.

During the acclimatization period, the males and females will be housed in groups of about 4 per sex, in suspended stainless steel group cages (45x32x18 cm) with wire mesh floor and front. For mating one male and two females will be housed together in smaller suspended wire mesh cages (18x32x18 cm) with wire mesh floor and front. Mated F0-females will be housed individually in suspended wire mesh cages (18x32x18 cm) with wire mesh floor and front. After the mating period, non-mated females will be housed individually until sacrifice and males will be returned to their group cage.

Shortly before littering, the females will be housed in macrolon cages (45x32x18 cm) with sterilized dust-free saw dust and wood shavings as nesting material; during the lactation period the females and their litters will remain in these cages.

At or shortly after PN day 21, F1-animals will be housed in groups of about 4 per sex, in suspended stainless steel group cages (45x32x18 cm) with wire mesh floor and front.

## 8.2 Feed and drinking water

Feed and water will be provided *ad libitum* from the arrival of the rats until the end of the study.

The rats will be fed a commercial rodent diet (Rat & Mouse No. 3 Breeding diet, RM3). For this study one batch will be used, which is analyzed by the supplier (SDS Special Diets Services, Witham, England) for nutrients and contaminants. The certificate of analysis pertaining to the batch used in this study will be included in the study report. The feed will be provided as a powder, in stainless steel cans, covered by a perforated stainless steel plate that serves to prevent spillage. The feed in the feeders will be refreshed once per week and topped up when necessary.

During the quarantine and acclimatization period of the F0-animals and after weaning of the F1-generation, drinking water (tap-water) will be supplied in polypropylene bottles with rubber stoppers and stainless steel nipple, that will be cleaned about weekly and filled up when necessary. Tap water suitable for human consumption (quality guidelines according to Dutch legislation based on EEC Council Directive 80/778/EEC) will be supplied by N.V. Waterleiding bedrijf Midden-Nederland. Results of the routine physical, chemical and microbiological examination of drinking water as conducted by the supplier will be made available to TNO Nutrition and Food Research Institute. In addition, the supplier periodically (twice per year) analyzes water samples taken at the premises of TNO Nutrition and Food Research Institute in Zeist for a limited number of physical, chemical and microbiological variables. The results of the samples taken during or close to the conduct of this study will be presented in the study report.

During the treatment period, drinking water (Milli-Q water or solutions of butyl benzyl phthalate or DES in Milli-Q water) will be provided in glass bottles with teflon/teflon coated stoppers with stainless steel nipples (see section 9.2.2 for details on preparation and handling of test substance solutions).

## 9 Experimental procedures

### 9.1 Experimental schedules and mating procedure

After 2 weeks of treatment (pre-mating period), two females will be caged with one untreated male. If mating does not occur after 1 week, females will be housed with a different male for another week. Every consecutive morning during the mating period, vaginal smears will be made to ascertain copulation by detection of sperm cells in the smear. Upon evidence of copulation, females will be housed individually for the birth and rearing their young (F1-generation). The day a sperm-positive smear is detected will be considered as gestation day 0. If a male dies in the pre-mating period or in the mating period before the female is found positive, the female will be mated with a proven male (i.e. a male which already had a successful copulation (positive smear) with another female). A rest

period of at least 2 days will be taken between the mating periods of the male. The mating period for these pairs will be 1 week. Mating pairs will be clearly identified. Males will be used for mating purposes only and discarded after the mating period. Non-mated females will be sacrificed after the mating period. The morning after birth will be considered postnatal day 1 (PN day 1). Consequently, for litters that are born during the day, but after the morning observation, that day will be considered PN day 0. On PN day 4, litters of more than eight pups will be adjusted by eliminating extra pups by random selection to yield, as nearly as possible, 4 males and 4 females per litter. Pups killed at culling will be examined externally for abnormalities and subsequently preserved in a neutral aqueous phosphate buffered 4% solution of formaldehyde. On PN day 21, the F1-pups will be weaned. At least 20 litters will be selected at random from each group and reared for about another 10 weeks. These animals will be sacrificed and necropsied when they are 90-97 days old. The pups of the remaining F1-litters will be discarded after an external examination and subsequently preserved in a neutral aqueous phosphate buffered 4% solution of formaldehyde. The F0-females will be sacrificed and necropsied after weaning.

## **9.2 Administration of the test substance**

### **9.2.1 Route and duration of administration**

The oral route will be used because this is an anticipated route of exposure in humans. F0-females will be exposed to the test substance in their drinking water for 2 weeks prior to mating, throughout mating and gestation periods and during the lactation period until sacrifice. F0-males will receive the test substance in their drinking water during the mating period only. After weaning, the F1-animals will receive drinking water (tap water) without added test substance (BBP) or DES.

### **9.2.2 Preparation and handling of the test substance solutions**

Fresh stock solutions of the BBP in ethanol (100, 1000 and 3000 mg BBP/ l ethanol) will be prepared once monthly. All BBP dosing solutions will be prepared daily from the stock solution by adding 1 ml stock solutions to 1 l Milli-Q water. The control dosing solution (1 l Milli-Q water to which 1 ml ethanol is added) will also be prepared daily.

A stock solution of DES in ethanol (50 mg/l ethanol) will be prepared once before the start of the study.

The dosing solution containing DES will be prepared at least once weekly. Drinking bottles containing DES solutions will be filled up when necessary and refreshed once weekly.

All glassware used in the preparation of dosing solutions and in administration to the test animals will be assigned permanently to the specific treatment group and reused as appropriate to prevent loss of test substance through adsorption to the

container walls and stoppers. Drinking bottles assigned to the BBP groups (B,C and D) will be filled and stored for at least 24 hours with the BBP dosing solutions before being used in the study. After use for a week the drinking bottles will be cleaned.

### 9.3 Experimental groups and dose levels

The study will comprise five groups, viz. three test groups receiving different levels of butyl benzyl phthalate (BBP), one vehicle control group and one positive control group (diethylstilbestrol, DES). The dose levels were selected on the basis of effects of BBP in the study of Sharpe et al. (1995) (mid dose) and the maximum solubility in water (high dose). Furthermore, an additional low dose was selected. Based on confidential data of the sponsor the dose level of DES was selected.

Each group of F0-females will consist of 28 animals. For mating 14 males will be allocated to each group. The various groups are presented in the scheme below.

Group	Treatment	Colour code	Dose level	Number of females	Number of males
			$\mu\text{g/litre}$		
A	control <sup>1</sup>	white	0	28	14
B	BBP	blue	low (100)	28	14
C	BBP	green	mid (1000)	28	14
D	BBP	red	high (3000) <sup>2</sup>	28	14
E	DES	yellow	50	28	14

<sup>1</sup> Milli-Q water containing 0.1 % ethanol

<sup>2</sup> the highest soluble dose will be used

### 9.4 Observations, analyses and measurements

#### 9.4.1 BBP analyses in stock solutions

The concentration of BBP in stock solutions, 3 in total during the administration period, will be checked immediately after preparation and near the end of their use. This will be done using UV spectrophotometric analysis at a single wavelength of the stock solution and the solvent (ethanol). The concentration will be calculated from the extinction difference and the molar extinction coefficient. Preferably, the molar extinction coefficient will be provided by the sponsor if available. Otherwise, the molar extinction coefficient will be determined. For acceptance of a BBP stock solution the measured concentration

should be within 97.5 to 102.5% of the theoretical concentration.

#### 9.4.2 BBP analyses in Milli-Q water

##### Method development

A specific method will be developed for the determination of BBP in the dosing solutions. The concentration range of BBP in the dosing solutions is 100 to 3000  $\mu\text{g/l}$ . However, the method will be used to monitor the presence of BBP at concentrations in drinking water without added BBP well below 100  $\mu\text{g/l}$ . Therefore, the LOQ (lower limit of quantification) and LOD (lower limit of detection) should preferably be in the order of several  $\mu\text{g/l}$ .

The method of choice is LC-MS using atmospheric pressure chemical ionisation interface (APCI). Selected ion monitoring mode (SIM mode) will be applied to monitor the protonated molecules of BBP and a suitable internal standard (e.g. deuterated dibutylphthalate). Depending on the LOD and LOQ required, MS-MS will be applied in SRM mode to increase the sensitivity. Using injections of c. 200  $\mu\text{l}$  of drinking water, the total amount of BBP injected is 200  $\mu\text{g}$  for a concentration of 1  $\mu\text{g/l}$ . This sensitivity is amenable with LC-MS(-MS). For accurate determinations an internal standard is mandatory. An isomer of BBP, isobutyl benzyl phthalate, will be evaluated as possible internal standard.

##### Method validation

Method validation will consist of assessing the performance characteristics of the method for the determination of BBP in dosing solutions. The following performance characteristics will be assessed:

- LOD (the concentration for which the s/n ratio is 3, obtained by extrapolation)
- LOQ (the lowest concentration of the QC samples resulting in an imprecision and bias <10%)
- calibration model and weight factor
- intra-occasion precision (n=3, number of replicates per QC)
- inter-occasion precision (n=3, number of occasions)
- accuracy (or bias, n=3, number of replicates per QC)

Calibration standards in drinking water containing BBP at 10, 50, 100, 500, 1000 and 3000  $\mu\text{g/l}$  will be prepared and analysed (n=1) on three occasions. On these occasions, quality control (QC) samples consisting of drinking water containing BBP at concentrations of 10, 30, 100, 300, 1000 and 3000  $\mu\text{g/l}$  will be analysed (n=3). In addition, a blank drinking water will be analysed on each occasion in triplicate.

The results obtained for the calibration standards will be used to select the best calibration model (weight factor). The QC results will be evaluated using single factor ANOVA.

The method validation should prove that the accuracy for a single analysis of any dosing solution is within 90 to 110% of the actual concentration.

#### Sample analysis

Each run will consist of the analysis of calibration standards (see method validation), QC samples (10, 100 and 1000  $\mu\text{g/l}$ ,  $n=2$ ) and authentic samples. The run will be rejected if for more than 2 QC results a bias of more than 10% is obtained.

The bulk BBP dosing solutions will be tested for homogeneity by taking samples at 3 positions, i.e. top, middle and bottom, of the containers. At the start of the administration period, 5 bottles per dosing group will be analysed prior to administration. In addition, each week during the administration period 5 bottles per dose levels will be analysed post administration. Blank dosing solutions will be analysed weekly throughout the study.

### **9.4.3 DES analyses in Milli-Q water**

#### Method development

A specific method will be developed for the determination of DES in the dosing solutions. The concentration of DES in the dosing solutions is c. 50  $\mu\text{g/l}$ .

The method of choice is direct analysis, large volume injections, of dosing solution by means of HPLC with UV-detection.

#### Method validation

Method validation will consist of assessing the performance characteristics of the method for the determination of DES in dosing solutions. The following performance characteristics will be assessed:

- intra-occasion precision ( $n=3$ , number of replicates per QC)
- inter-occasion precision reproducibility ( $n=3$ , number of occasions)
- accuracy (or bias,  $n=3$ , number of replicates per QC)

Calibration standards in drinking water containing DES at 25, 50 and 75  $\mu\text{g/l}$  will be prepared and analysed ( $n=1$ ) on three occasions. On these occasions, quality control (QC) samples consisting of drinking water containing DES at a concentration of 50  $\mu\text{g/l}$  will be analysed ( $n=3$ ).

The QC results will be evaluated using single factor ANOVA.

The method validation should prove that the accuracy for a single analysis of the dosing solution is within 90 to 110% of the actual concentration.

### Sample analysis

Each run will consist of the analysis of calibration standards, QC samples (50  $\mu\text{g/l}$ ,  $n=2$ ) and authentic samples. The run will be rejected if for more than 1 QC result a bias of more than 10% is obtained. At the start and in week 5 of the administration period the bulk DES solution will be analysed prior to administration. In addition, in the first week of the administration period 5 bottles will be analysed post-administration. The control dosing solution (0  $\mu\text{g/l}$  BBP) will be tested for contamination with DES at the start of the study and in week 5.

#### **9.4.4 Clinical signs**

Each animal will be observed daily in the morning hours by careful observations. On working days, all cages will be checked again in the afternoon. On Saturdays, Sundays and public holidays only one check per day will be carried out. All abnormalities, signs of ill health or reaction to treatment will be recorded. Any animal showing signs of severe debility or intoxication, particularly if death appears imminent, will be killed by exsanguination to prevent loss of tissues by cannibalism or autolytic degeneration.

#### **9.4.5 Body weight**

##### *F0-females*

Body weights of female rats will be recorded on day -3 (randomization) weekly during the pre-mating and mating periods, on days 0, 7, 14 and 21 of gestation, and during lactation on PN days 1, 7, 14 and 21.

Body weight of mated females which produce no litter will be recorded up to day 21 of the presumed gestation period.

##### *F1-animals*

After weaning body weights of the F1-animals will be recorded weekly. All animals will be weighed at sacrifice.

#### **9.4.6 Food consumption**

The quantity of food consumed by the animals will be measured on a cage basis, by weighing the feeders. In the report the food consumption will be presented per week (g/cage) in the appendices and expressed in g/kg body weight/day in the summarizing tables.

##### *F0-females*

Food consumption of females will be recorded weekly during the pre-mating and gestation periods, and in the lactation period from PN days 1-7, 7-14, 14-21.

Food consumption of mated females which produce no litter will be recorded up to day 21 of the presumed gestation period.

*F1-animals*

Food consumption will be measured weekly.

**9.4.7 Water consumption**

The quantity of water consumed by the animals will be measured on a cage basis, by weighing the drinking bottles daily. In the report the water consumption will be presented per week (g/cage) in the appendices and expressed in g/kg body weight/day in the summarizing tables

*F0-females*

Water consumption of females will be measured over successive 7-day periods during the pre mating and gestation periods and from PN days 1-7, 7-14 and 14-21 during the lactation period.

Water consumption of mated females which produce no litter will be recorded up to day 21 of the presumed gestation period.

*F1-animals*

Water consumption will not be measured.

**9.4.8 Parturition and litter evaluation**

At the end of the gestation period, females will be examined twice daily for signs of parturition. Any difficulties occurring during parturition will be recorded.

To keep nest disturbance to a minimum the litters will be examined only daily for dead pups.

The total litter size and numbers of each sex as well as the numbers of stillbirths, livebirths and grossly malformed pups and pups showing abnormalities will be recorded on PN days 1, 4, 7, 14 and 21. Grossly malformed pups will be sacrificed and examined by appropriate techniques.

**9.4.9 Pup weight**

The litters will be weighed on PN days 1, 4 (before and after culling), 7 and 14. At weaning (PN day 21), all pups will be weighed individually.

**9.4.10 Sexual maturation**

The following landmarks for physical development will be recorded in the F1-animals:

- Males: preputial separation from PN day 29.
- Females: vaginal opening from PN day 31.

#### **9.4.11 Oestrus cycle length**

Vaginal smears will be made for 14 consecutive days to evaluate the oestrus cycle length in the F1-females, when they are about 10 weeks of age.

#### **9.4.12 Sampling of urine of F0-females**

At the end of the weaning period (PN day 21), 5 dams from the control and BBP-groups will be placed in stainless steel metabolism cages (1 rat/cage) for 2 consecutive days. The first day serves to acclimatize the animals to the metabolism cage. On the second day, 24-hour urine samples will be collected. The animals will have free access to food and drinking water containing the test substance with every precaution to prevent cross-contamination of the urine samples. The urine samples will be collected in vials (provided by the Sponsor) kept cold in insulated containers filled with ice. The weight of each vial will be measured before and after sampling. Vials containing urine will be stored at -20°C until shipment to the Sponsor.

#### **9.4.13 Gross necropsy of F1-pups and weanlings**

All stillborn pups, pups found dead and pups that are terminated in a moribund condition during the study will be examined macroscopically for structural abnormalities or pathological changes. Gross necropsy will also be conducted on pups of dams that die during lactation (these pups will be sacrificed at the time of the dam's death). Pups not selected for further examination will be discarded after external examination.

All pups will be preserved in a neutral aqueous phosphate buffered 4% solution of formaldehyde.

#### **9.4.14 Gross necropsy of F0-animals**

After mating F0-males will be sacrificed by CO<sub>2</sub> and discarded without further necropsy.

All surviving F0-females will be subjected to a thorough necropsy. They will be killed by decapitation under ether anaesthesia after weaning of their litter (females with live litter), after the mating period (non-mated females), after day 23 of the presumed gestation (non-pregnant females), or at a time point to be decided by the Study director (females without live litters).

Gross necropsy will also be performed on animals that die intercurrently (if not precluded by autolysis or cannibalism) or that have to be killed because they are moribund. An effort will be made to determine the probable cause of death.

From all F0-females the uterus will be examined for the presence of implantation sites and organs and tissues showing abnormalities will be preserved in neutral, aqueous phosphate buffered 4% solution of formaldehyde.

In addition, at weaning from all F0-females with live litters the organs listed below will be preserved in neutral, aqueous phosphate buffered 4% solution of formaldehyde:

- uterus including cervix
- ovaries
- vagina
- liver
- pituitary
- adrenal glands
- hypothalamus
- organs or tissues showing macroscopic abnormalities

#### 9.4.15 Gross necropsy of F1-animals

Gross necropsy will be performed on animals that die intercurrently (if not precluded by autolysis or cannibalism) or that have to be killed because they are moribund. An effort will be made to determine the probable cause of death. At sacrifice animals will be killed by decapitation under ether anaesthesia. F1-animals will be killed when they are 90-97 days old.

For the F1-generation males the following organs will be preserved and the underlined organs will be weighed:

- testes\* (left and right testis will be weighed separately, left testis will be used for the determination of sperm parameters)
- epididymides\* (left and right epididymis together, in addition the left cauda epididymis will be weighed separately and used for the determination of sperm parameters)
- seminal vesicles (with coagulating glands and their fluids)\*
- prostate\*
- liver
- kidneys
- adrenal glands\*
- thyroid\*
- pituitary\*
- hypothalamus\*
- organs or tissues showing macroscopic abnormalities

For the F1-generation females the following organs will be preserved and the underlined organs will be weighed:

- ovaries\*
- uterus and cervix \*
- vagina\*
- liver
- kidneys
- adrenal glands\*
- thyroid\*

- pituitary\*
- hypothalamus\*
- organs or tissues showing macroscopic abnormalities

Organs or tissues showing macroscopic abnormalities and all organs marked with a \* will be preserved in a neutral, aqueous phosphate buffered 4% solution of formaldehyde or in Bouin's fixative (testes only) for possible further examination.

#### **9.4.16 Sperm parameters**

Epididymal sperm will be derived from the left cauda epididymis and the motility will be measured with the Hamilton Thorne Integrated Visual Optical System (IVOS). For this purpose the cauda epididymis will be dissected, weighed and thereafter minced in M199 medium containing 0.5% BSA. From this sperm sample the cauda epididymal sperm reserves will also be enumerated with the IVOS. A smear of the sperm solution will be stained and two hundred spermatozoa will be evaluated for morphological abnormalities.

At necropsy the left testis will be stored on dry ice and frozen at - 80°C for later evaluation of the number of homogenisation-resistant spermatids. Before analysis the testis will be thawed and the tunica albuginea will be removed. After weighing, the testicular parenchyme will be minced and homogenated in Saline Triton X-100 solution. The homogenization resistant sperm heads will be enumerated using the IVOS.

#### **9.4.17 Fertility and reproductive performance**

The following data will be presented for each group:

- number of females placed with males
- number of successful copulations (= number of females mated)
- number of pregnant females
- number of females surviving delivery
- number of females with liveborn and (all) dead embryos
- number of females with liveborn and (all) stillborn pups
- number of pups delivered (live- and stillborn)
- number of live pups at day 1, 4, 7, 14 and 21
- number of pups lost during days 1-4, 5-7, 8-14 and 15-21
- number of litters lost entirely during days 1-4, 5-7, 8-14, 15-21 and 0-21
- number of pups culled
- number of pups alive after culling
- number of male pups at day 1 and 21
- number of implantation sites
- number of lost implantation sites

The following parameters will be calculated:

- pre-coital time = time between the start of mating and successful copulation

- duration of gestation = time between gestation day 0 and day of delivery
- mating index = (number of females mated/number of females placed with males) x 100
- female fertility index = (number of pregnant females/number of females placed with males) x 100
- female fecundity index = (number of pregnant females/number of males mated) x 100
- gestation index = (number of females with live pups/number of pregnant females) x 100
- live birth index = (number of pups born alive/number of pups born) x 100
- viability index (days 4-21) = (number of live weanlings/number of pups alive on day 4 post partum after culling) x 100
- pup mortality day 1 = (number of dead pups on day 1/total number of pups on day 1) x 100
- sex ratio day n = (number of live male pups on day n/ number of live pups on day n) x 100
- post-implantation loss = [(number of implantation sites - number of pups born (embryos) alive)/number of implantation sites] x 100

## 10 Statistical analysis of the results

Statistical procedures used in the evaluation of data are generally as follows:

- for (pup) body weights and food consumption: one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests
- for clinical signs: Fisher's exact probability test
- for pre-coital time and duration of gestation: Kruskal-Wallis followed by Mann-Whitney U-tests
- for number of females pregnant, females with liveborn, females surviving delivery, females with (all) stillborn pups, number of live- and stillborn pups, number of pups/litters lost, number of male pups and number of implantation sites: Fisher's exact probability test
- for mean number of pups delivered, mean number of pups alive, mean number of implantations and post-implantation loss: Kruskal-Wallis followed by Mann-Whitney U-tests
- preputial separation and vaginal opening: ANOVA followed by Dunnett's multiple comparison tests
- for oestrus cycle length: ANOVA followed by Dunnett's multiple comparison tests
- for organ weights (absolute and relative): ANOVA followed by Dunnett's multiple comparison tests
- for sperm parameters: ANOVA followed by Dunnett's multiple comparison tests
- for pathological changes: Fisher's exact probability test

Other statistical tests will be performed when considered appropriate. All tests

will be two-sided. As a level of significance will be considered  $P < 0.05$ .

## **11 Retention of records, samples and specimens**

A reference sample of the test substance will be retained for ten years if its nature allows this. Unless otherwise agreed, remaining test substance will be retained for at least six months after submission of the final report. Raw data, the master copy of the final report and all other information relevant to the quality and integrity of the study, including tissue specimens, paraffin blocks and microscopic slides, will be retained in the archives of TNO Nutrition and Food Research Institute for a period of at least five years (tissue specimens, paraffin blocks) or at least 15 years (slides, raw data) after reporting of the study. At the end of the five year storage period, the Sponsor will be asked whether the tissue specimens and paraffin blocks can be discarded, should be stored for an additional period, or transferred to the archives of the Sponsor.

## **12 Reporting**

During the study summarizing tables (not QA-audited) will be made available shortly after the mating and after the weaning period. Before issuing a final report, a draft report will be sent to the sponsor.

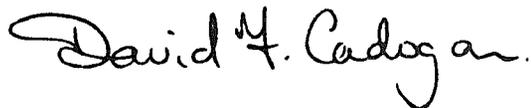
A final report will be prepared including details about the objective of the study, characterization and administration of the test substance, testing facility, responsible personnel, dates on which the study was initiated and completed, the test system, observations and measurements, materials and methods, statistical methods, evaluation of the results and, where appropriate, discussion and conclusions, and the location of the raw data and the final report. The final report will contain a statement by the Quality Assurance Unit and a statement on GLP compliance signed by the study director. Results will be presented in summarizing tables. Individual data will be presented in appendices.

## **13 Quality assurance**

The Quality Assurance Unit of TNO Nutrition and Food Research Institute will inspect the ongoing study and the raw data and review the final report as required by the OECD Principles of Good Laboratory Practice. The statement of the Quality Assurance Unit will specify the dates of inspections and reports to management and to the study director. Representatives of the sponsor or regulatory authorities may conduct additional inspections of the testing facility and/or the raw data.

## 14 Approval of the protocol

For the Sponsor:



Dr D.F. Cadogan  
(European Council for Plasticizers and Intermediates)

Date: 24/4/97

For TNO Nutrition and Food Research Institute:



Dr E.R. Verheij  
(Principal Investigator, Analytical Science Division)

Date:

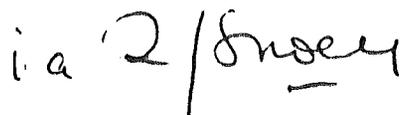
April 15, 1997



Ir D.H. Waalkens-Berendsen  
(Study director)

Date:

April 18, 1997



Dr B.M. Kulig  
(Management)

Date:

April 18, 1997

## 15 Annexes

The following documents are attached to this protocol:  
Annex 1: Distribution list

### **Annex 1 - Distribution list**

Testing facility: Sponsor  
Assistant study director  
Archivist  
Head animal care  
Deputy study director  
Pathology (2x)  
Principal Investigator, Analytical Science Division (2x)  
Study director  
Quality Assurance Unit

Attachment III, Table 1

STUDY NO.1899F1 ORAL DEVELOPMENTAL REPRODUCTION STUDY WITH BUTYL BENZYL PHTHALATE IN WISTAR RATS

TABLE:35.2 MEAN ORGAN WEIGHTS RELATIVE TO TERMINAL BODY WEIGHT (g/kg b.w.)

	MALES				
	A CONTROL	B LOW DOSE BBP	C MID DOSE BBP	D HIGH DOSE BBP	E DES DES
TERMINAL BODY WEIGHT (g)	MEAN 349.6 d S.E. 3.44 N 78	345.0 3.66 82	351.3 3.53 78	346.2 4.13 75	323.2# 3.47 56
TESTIS (L)	MEAN 4.476 d S.E. 0.0528 N 77	4.495 0.0638 81	4.330 0.0647 78	4.357 0.0834 75	4.557 0.0629 56
TESTIS (R)	MEAN 4.427 d S.E. 0.0585 N 78	4.492 0.0628 82	4.281 0.0751 78	4.331 0.0790 75	4.499 0.0594 56
EPIDIDYIMIDES	MEAN 3.217 d S.E. 0.0322 N 78	3.229 0.0390 81	3.181 0.0325 78	3.174 0.0450 75	3.323 0.0357 56
CAUDA EPIDIDYIMIS, LEFT	MEAN 0.675 d S.E. 0.0090 N 77	0.665 0.0091 81	0.667 0.0072 78	0.655 0.0120 75	0.692 0.0103 56
SEMINAL VESICLES	MEAN 3.014 d S.E. 0.0660 N 78	2.887 0.0800 82	3.029 0.0680 78	2.953 0.0716 75	3.129 0.0861 56
PROSTATE	MEAN 2.438 d S.E. 0.0405 N 78	2.351 0.0482 82	2.373 0.0490 78	2.344 0.0429 75	2.614 0.0671 56
LIVER	MEAN 37.559 d S.E. 0.3347 N 78	37.994 0.3914 82	38.812 0.3429 78	37.758 0.4108 75	39.786# 0.3929 56
KIDNEYS	MEAN 5.620 d S.E. 0.0529 N 78	5.569 0.0487 82	5.794 0.0500 78	5.736 0.0554 75	5.844* 0.0594 56

Statistical key: d= ANOVA & Dunnett test \* = p<0.05 # = p<0.001

STUDY NO.1899F1 ORAL DEVELOPMENTAL REPRODUCTION STUDY WITH BUTYL BENZYL PHTHALATE IN WISTAR RATS

TABLE:35.1 MEAN ORGAN WEIGHTS ABSOLUTE (g)

MALES

		A	B	C	D	E
		CONTROL	LOW DOSE BBP	MID DOSE BBP	HIGH DOSE BBP	DES
TERMINAL BODY WEIGHT	MEAN	349.6 d	345.0	351.3	346.2	323.2#
	S.E.	3.44	3.66	3.53	4.13	3.47
	N	78	82	78	75	56
TESTIS (L)	MEAN	1.556 d	1.551	1.514	1.512	1.465*
	S.E.	0.0131	0.0210	0.0217	0.0307	0.0154
	N	77	81	78	75	56
TESTIS (R)	MEAN	1.538 d	1.549	1.497	1.502	1.447*
	S.E.	0.0163	0.0205	0.0254	0.0293	0.0151
	N	78	82	78	75	56
EPIDIDYMIDES	MEAN	1.121 d	1.116	1.115	1.098	1.071
	S.E.	0.0118	0.0149	0.0132	0.0178	0.0118
	N	78	81	78	75	56
CAUDA EPIDIDYMIS, LEFT	MEAN	0.236 d	0.230	0.234	0.227	0.223
	S.E.	0.0034	0.0034	0.0029	0.0044	0.0032
	N	77	81	78	75	56
SEMINAL VESICLES	MEAN	1.051 d	0.995	1.063	1.022	1.007
	S.E.	0.0231	0.0266	0.0246	0.0248	0.0270
	N	78	82	78	75	56
PROSTATE	MEAN	0.848 d	0.811	0.832	0.810	0.844
	S.E.	0.0128	0.0169	0.0178	0.0160	0.0220
	N	78	82	78	75	56
LIVER	MEAN	13.119 d	13.169	13.636	13.116	12.862
	S.E.	0.1616	0.2204	0.1846	0.2204	0.1928
	N	78	82	78	75	56
KIDNEYS	MEAN	1.961 d	1.915	2.030	1.979	1.885
	S.E.	0.0230	0.0189	0.0216	0.0228	0.0226
	N	78	82	78	75	56

Statistical key: d= ANOVA & Dunnett test \* = p<0.05 # = p<0.001

# Attachment III, Table 2

STUDY NO. 1899F1M

ORAL DEVELOPMENTAL REPRODUCTION STUDY WITH  
BUTYL BENZYL PHTHALATE IN WISTAR RATS  
(F1-GENERATION; MALES)

TABLE 40

SUMMARY TESTICULAR SPERM COUNTS

MALES

		GROUP A CONTROL	GROUP B LOW DOSE BBP	GROUP C MID DOSE BBP	GROUP D HIGH DOSE BBP	GROUP E DES
Testicular parenchyma weight (gram)	MEAN	1.361 <sup>d</sup>	1.343	1.316	1.323	1.263**
	S.E.	0.013	0.019	0.020	0.028	0.016
	N	77	81	78	75	56
No. of spermatozoa in 25 ml • 10 <sup>5</sup>	MEAN	145.8 <sup>d</sup>	145.6	147.1	137.9	136.4
	S.E.	5.2	4.6	5.2	5.0	4.7
	N	77	81	78	75	56
No. of spermatozoa per g testicular parenchyma • 10 <sup>6</sup>	MEAN	106.7 <sup>d</sup>	107.0	110.0	102.0	108.0
	S.E.	3.5	3.1	3.5	3.3	3.6
	N	77	81	78	75	56
Daily sperm production <sup>(1)</sup> • 10 <sup>5</sup>	MEAN	17.5 <sup>d</sup>	17.5	18.0	16.7	17.7
	S.E.	0.6	0.5	0.6	0.5	0.6
	N	77	81	78	75	56

Statistical key: <sup>d</sup> = ANOVA & DUNNET

\*\* = P < 0.01

(1) No of spermatozoa per g. testicular parenchyma / 6.10

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Attachment III, Table 3

STUDY NO.1899F0 ORAL DEVELOPMENTAL REPRODUCTION STUDY WITH BUTYL BENEYL PHTHALATE IN WISTAR RATS (F0-GENERATION)

TABLE:18 NATURAL DELIVERY DATA AND LITTER DATA -- SUMMARY

	A	B	C	D	E
	CONTROL	LOW DOSE BBP	MID DOSE BBP	HIGH DOSE BBP	DES
Females placed with males	N 28	28	28	28	28
Females mated	N 26	28	26	28	28
Pre-coital time (days)	MEAN 3.19 u	3.43	2.73	3.00	3.18
	S.E. 0.532	0.612	0.204	0.477	0.309
Day 1 to 4	N 23 f	25	26	25	22
	88	89	100	89	79
Day 5 to 7	N 1 f	1	0	2	6
	3.8	3.6	0.0	7.4	21
Day 8 to 14	N 2 f	2	0	1	0
	7.7	7.1	0.0	3.7	0.0
Females pregnant	N 25 f	23	23	24	21
Females with liveborn	N 24 f	23	22	24	20
Mating index	93	100	93	100	100
Female fecundity index	96	82	88	86	75
Female fertility index	89	82	82	86	75
Gestation index	96	100	96	100	95
Duration of gestation	MEAN 21.36 u	21.26	21.59	21.46	21.71*
	S.E. 0.090	0.113	0.126	0.120	0.101
Females surviving delivery as % of pregnant females	N 25 f	23	22	24	21
	100	100	96	100	100
Females with stillborn pups as % of pregnant females	N 4 f	0	0	5	3
	16	0.0	0.0	21	14
Females with all stillborn pups as % of pregnant females	N 1 f	0	0	0	1
	4.0	0.0	0.0	0.0	4.8

Statistical key: f= Fishers exact test u= Kruskal-Mallis & Mann-Whitney U \* = p<0.05

STUDY NO. 1099F0 ORAL DEVELOPMENTAL REPRODUCTION STUDY WITH BUTYL BENZYL PHTHALATE IN WISTAR RATS (F0-GENERATION)

TABLE 10 NATURAL DELIVERY DATA AND LITTER DATA -- SUMMARY

	A CONTROL		B LOW DOSE BBP		C MID DOSE BBP		D HIGH DOSE BBP		E DES	
Pups delivered (total)	N	252	N	233	212	248	179			
	MEAN	10.08 u	10.13	9.64	10.33	10.33	0.52**			
	S.E.	0.412	0.384	0.590	0.416	0.328				
Liveborn	N	237 f	233#	212#	241	166				
Live Birth Index	%	94	100	100	97	93				
Stillborn	N	15 f	0#	0#	7	13				
Pup mortality day 1	%	6.0	0.0	0.0	2.8	7.3				
Number of pups lost (dying, missing, and/or cannibalized) on the following days:										
days 1-4	N	2 f	2	30#	29#	39#				
Pup mortality day 4	%	0.8	0.9	14	12	23				
Called day 4	N	49	51	39	43	10				
Number alive, after culling at day 4	N	186	180	143	169	117				
Number of pups lost (dying, missing, and/or cannibalized) on the following days:										
days 5-7	N	1 f	1	0	1	0				
days 8-14	N	0 f	0	0	0	0				
days 15-21	N	0 f	0	0	0	0				
Pups alive day 21	N	185 f	171 b	143	168	117				
Viability index day 4-21	%	99	99	100	99	100				
Number of litters lost entirely (Stillborn, dying, missing, cannibalized, and/or culled) in the period between:										
days 0-4	N	1 f	0	3	1	5				
	%	4.2	0.0	14	4.2	25				
days 5-7	N	0 f	0	0	0	0				
	%	0.0	0.0	0.0	0.0	0.0				
days 8-14	N	0 f	0	0	0	0				
	%	0.0	0.0	0.0	0.0	0.0				
days 15-21	N	0 f	0	0	0	0				
	%	0.0	0.0	0.0	0.0	0.0				
days 0-21	N	1 f	0	3	1	5				
	%	4.2	0.0	14	4.2	25				

Statistical key: f= Fishers exact test u= Kruskal-Wallis & Mann-Whitney U \* = p<0.05 \*\* = p<0.01 # = p<0.001  
 b = after sacrifice of female B93 and 8 pups on day 15 of lactation

STUDY NO. 1899F0 ORAL DEVELOPMENTAL REPRODUCTION STUDY WITH BUTYL BENZYL PHTHALATE IN Wistar rats (F0-GENERATION)

TABLE: 18 NATURAL DELIVERY DATA AND LITTER DATA -- SUMMARY

	A CONTROL		B LOW DOSE BBP		C MID DOSE BBP		D HIGH DOSE BBP		E DMS	
Live Pups/litter										
day 1	MEAN	9.88 u	10.13	9.64	10.04	10.04	9.30**			
	S.E.	0.405	0.304	0.590	0.436		0.378			
day 4 preculling	MEAN	9.79 u	10.04	9.58	9.22	7.94**				
	S.E.	0.413	0.415	0.564	0.562	0.442				
day 4 postculling	MEAN	7.75 u	7.83	7.53	7.35	7.31				
	S.E.	0.150	0.174	0.290	0.336	0.254				
day 7	MEAN	7.71 u	7.78	7.53	7.30	7.31				
	S.E.	0.153	0.177	0.290	0.364	0.254				
day 14	MEAN	7.71 u	7.78	7.53	7.30	7.31				
	S.E.	0.153	0.177	0.290	0.364	0.254				
day 21	MEAN	7.71 u	7.77	7.53	7.30	7.31				
	S.E.	0.153	0.185	0.290	0.364	0.254				
Number of male pups at day 1	N	121 f	125	106	126	86				
Sex ratio at day 1	♂	51	54	50	52	52				
Number of male pups at day 21	N	93 f	91 b	78	87	56				
Sex ratio at day 21	♂	50	53	55	52	48				
Post implantation loss	N	45	24	36	30	32				
% impl. per animal	MEAN	16.22 u	9.33	13.87	11.34	15.62				
	S.E.	4.273	1.883	4.421	2.755	5.042				

Statistical key: f = Fishers exact test u = Kruskal-Wallis & Mann-Whitney U \*\* = p<0.01  
 b = after sacrifice of B93 on day 15 of lactation

Mating index : number of females mated \* 100 / number of females placed with males  
 Female fecundity index : number of females pregnant \* 100 / number of females mated  
 Female fertility index : number of females pregnant \* 100 / number of females placed with males  
 Gestation index : number of females with live pups \* 100 / number of pregnant females  
 Live birth index : number of pups born alive \* 100 / total number of pups born  
 Pup mortality day n : number of dead pups on day n \* 100 / total number of pups on day n  
 Viability index day 4-21: number of pups surviving 21 days / number of liveborn after culling at day 4  
 Sex ratio : number of male pups date n \* 100 / total number of pups  
 Post-implantation loss : number of implantation sites - number of pups born alive \* 100 / number of implantation sites

First Mating - Data on a litter basis - *Data summarized, raw data follows*

	Control	0.1 ppm	1.0 ppm	2.5 ppm
# of litters with at least one stillborn pup	4 /25	0/23	0/23	5/24
# of litters with postnatal mortality	2/25	2/23	6/23	4/24

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STUDY NO. 1099E0 ORAL DEVELOPMENTAL REPRODUCTIVE STUDY WITH BUTYL BENZYL PHTHALATE IN WISTAR RATS (F0-GENERATION)

APPENDIX:19 DELIVERY AND LITTER DATA GROUP A: CONTROL

FEMALES#	LITTER DELIVERED				NUMBER OF LIVE PUPS												IMPLANTATION STATIONS		DURATION OF GESTATION (DAYS)		
	LIVE		DEAD		TOTAL		DAYS													M	F
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F					
3 SK	12	0	12	0	8	4	8	4	4	4	4	4	4	4	4	4	4	14	21		
5 SK	10	0	10	0	6	4	6	4	4	4	4	4	4	4	4	4	4	11	21		
7 SK	11	0	11	0	9	2	9	2	6	2	6	2	6	2	6	2	6	11	21		
9 SK	11	0	11	0	2	9	2	9	2	6	2	6	2	6	2	6	2	12	21		
11 SK	9	0	9	0	4	5	3	5	3	5	2	5	2	5	2	5	2	10	22		
13 SK	9	1	10	1	3	6	3	6	3	5	3	5	3	5	3	5	3	12	21		
15 SK	13	0	13	0	7	6	7	6	4	4	4	4	4	4	4	4	4	13	21		
17 SK	10	0	10	0	6	4	6	4	4	4	4	4	4	4	4	4	4	11	22		
19 SK	12	0	12	0	7	5	7	5	4	4	4	4	4	4	4	4	4	12	22		
21 SK	11	0	11	0	9	2	9	2	6	2	6	2	6	2	6	2	6	11	21		
23 PIK	0	11	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	22		
25 SK	0	0	0	0	5	3	5	3	5	3	5	3	5	3	5	3	5	10	22		
27 SK	11	0	11	0	2	9	2	9	2	6	2	6	2	6	2	6	2	12	21		
29 SK	11	2	13	2	4	7	4	7	4	4	4	4	4	4	4	4	4	13	21		
31 SK	9	0	9	0	4	5	4	5	4	4	4	4	4	4	4	4	4	12	22		
33 SK	12	0	12	0	5	7	5	7	4	4	4	4	4	4	4	4	4	12	22		
35 SK	6	0	6	0	3	3	3	3	3	3	3	3	3	3	3	3	3	11	21		
37 SK	10	0	10	0	5	5	5	5	4	4	4	4	4	4	4	4	4	11	21		
39 SK	9	0	9	0	4	5	4	5	4	4	4	4	4	4	4	4	4	9	21		
41 SK	11	0	11	0	9	2	9	2	6	2	6	2	6	2	6	2	6	12	21		
43 SK	0	0	0	0	3	5	3	5	3	5	3	5	3	5	3	5	3	9	22		
45 NOT MATED																					
47 SK	5	0	5	0	1	4	1	4	1	4	1	4	1	4	1	4	1	7	21		
49 SK	7	0	7	0	6	1	6	1	6	1	6	1	6	1	6	1	6	12	22		
51 SK	11	1	12	1	5	6	5	6	4	4	4	4	4	4	4	4	4	12	21		
53 SK	11	0	11	0	4	7	4	7	4	4	4	4	4	4	4	4	4	12	21		
55 NOT MATED																					

DAY 4 COLUMNS - PRE- AND POSTCULLING RESPECTIVELY

MF-NOT PREGNANT PIK-PREGNANT, INTERIM KILL NW-NOT MATED SK-SCHEDULED KILL

STUDY NO. 109990 ORAL DEVELOPMENTAL REPRODUCTION STUDY WITH  
 BUTYL BENZYL PHTHALATE IN WISTAR RATS  
 (F0-GENERATION)

APPENDIX:19 DELIVERY AND LITTER DATA  
 GROUP B; LOW DOSE BBP

FEMALES	LITTER DELIVERED			NUMBER OF LIVE PUPS												IMPLAN- TATIONS	DURATION OF GESTATION (DAYS)			
	LIVE DEAD TOTAL			1			4			7			14					21		
	M	F	M	M	F	M	F	M	F	M	F	M	F	M	F			M	F	
57 SK	13	0	13	5	0	5	0	4	4	4	4	4	4	4	4	4	4	13	21	
59 SK	11	0	11	7	4	7	4	4	4	4	4	4	4	4	4	4	4	11	21	
61 SK	0	0	0	4	4	4	4	4	4	4	4	4	4	4	4	4	4	9	21	
63 SK	11	0	11	4	7	4	4	4	4	4	4	4	4	4	4	4	4	12	21	
65 SK	10	0	10	5	5	5	5	4	4	4	4	4	4	4	4	4	4	12	21	
67NOT PREGNANT																				
69 SK	11	0	11	0	3	0	3	5	3	5	3	5	3	5	3	5	3	12	20	
71NOT PREGNANT																				
73 SK	12	0	12	6	6	6	6	4	4	4	4	4	4	4	4	4	4	12	21	
75 SK	13	0	13	0	5	0	5	4	4	4	4	4	4	4	4	4	4	14	21	
77 SK	9	0	9	4	5	4	5	4	4	4	4	4	4	4	4	4	4	11	21	
79 SK	10	0	10	4	6	4	6	4	4	4	4	4	4	4	4	4	4	11	21	
81 SK	13	0	13	5	0	5	0	4	4	4	4	4	4	4	4	4	4	13	21	
83 SK	11	0	11	6	5	6	5	4	4	4	4	4	4	4	4	4	4	12	21	
85 SK	5	0	5	5	0	4	0	4	0	4	0	4	0	4	0	4	0	6	22	
87 SK	10	0	10	5	5	5	5	4	4	4	4	4	4	4	4	4	4	12	22	
89 SK	10	0	10	6	4	5	4	4	4	4	4	4	4	4	4	4	4	10	21	
91NOT PREGNANT																				
93 PIK	10	0	10	7	3	7	3	5	3	5	3	5	3	5	3	5	3	13	21	
95 SK	10	0	10	5	5	5	5	4	4	4	4	4	4	4	4	4	4	10	21	
97 SK	10	0	10	6	4	6	4	4	4	4	4	4	4	4	4	4	4	10	22	
99 SK	10	0	10	7	3	7	3	5	3	5	3	5	3	5	3	5	3	10	21	
101 SK	8	0	8	5	3	5	3	5	3	5	3	5	3	5	3	5	3	12	22	
103 SK	9	0	9	3	5	3	5	3	5	3	5	3	5	3	5	3	5	9	22	
105 SK	9	0	9	6	3	6	3	5	3	5	3	5	3	5	3	5	3	11	22	
107NOT PREGNANT																				
109 SK	11	0	11	4	7	4	7	4	4	4	4	4	4	4	4	4	4	12	22	
111NOT PREGNANT																				

DAY 4 COLONES - P2E- AND POSTCOLLING RESPECTIVELY

BP-NOT PREGNANT PIK-PREGNANT, INTERIM KILL SK-SCHEDULED KILL  
 \*Animal no. 893 and pups sacrificed on day 15 of lactation

ReproTox version: 2.4

STUDY NO. 18999 ORAL DEVELOPMENTAL REPRODUCTION STUDY WITH  
 BUTYL METHYL FURYLATE IN WISTAR RATS  
 (F0-GENERATION)

APPENDIX:19 DELIVERY AND LITTER DATA  
 GROUP C: MID DOSE BDP

FEMALE	LITTER DELIVERED		NUMBER OF LIVE PUPS												IMPLAN- TATIONS	DURATION OF GESTATION (DAYS)	
	LIVE DEAD TOTAL		1		4		7		14		21						
	M	F	M	F	M	F	M	F	M	F	M	F					
113 SK	11	0	11	5	6	5	6	4	4	4	4	4	4	4	4	11	21
115 PIK	10	0	10	4	6	0	0	0	0	0	0	0	0	0	0	14	21
117NOT MATED	9	0	9	3	6	2	2	2	2	2	2	2	2	2	2	13	22
121 SK	12	0	12	4	8	4	4	4	4	4	4	4	4	4	4	12	21
123 SK	9	0	9	4	5	4	4	4	4	4	4	4	4	4	4	11	22
125 SK	4	0	4	1	3	1	3	1	3	1	3	1	3	1	3	5	22
127 SK	7	0	7	5	2	5	2	5	2	5	2	5	2	5	2	7	22
129 SK	11	0	11	4	7	4	4	4	4	4	4	4	4	4	4	12	22
131 SK	11	0	11	6	5	6	5	4	4	4	4	4	4	4	4	11	22
132NOT MATED	11	0	11	3	8	3	8	3	5	3	5	3	5	3	5	12	22
135 SK	11	0	11	8	3	8	3	5	3	5	3	5	3	5	3	12	22
137 SK	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	23
139 PIK	11	0	11	5	6	5	6	4	4	4	4	4	4	4	4	12	21
141 SK	12	0	12	5	7	0	0	0	0	0	0	0	0	0	0	13	21
143 PIK	9	0	9	4	5	4	5	4	4	4	4	4	4	4	4	12	21
145NOT PRECNANT	12	0	12	2	10	2	10	2	6	2	6	2	6	2	6	12	22
147 SK	8	0	8	6	2	6	2	6	2	6	2	6	2	6	2	10	21
149 SK	12	0	12	8	4	8	3	5	3	5	3	5	3	5	3	13	21
151 SK	12	0	12	8	4	8	3	5	3	5	3	5	3	5	3	13	21
153 SK	12	0	12	8	4	8	3	5	3	5	3	5	3	5	3	13	21
155NOT PRECNANT	12	0	12	8	4	8	4	4	4	4	4	4	4	4	4	12	21
157 SK	10	0	10	9	1	8	1	7	1	7	1	7	1	7	1	12	22
159 SK	8	0	8	6	2	6	2	6	2	6	2	6	2	6	2	9	22
161 SK	11	0	11	6	5	6	5	4	4	4	4	4	4	4	4	11	21
163NOT PRECNANT	11	0	11	6	5	6	5	4	4	4	4	4	4	4	4	11	21
165 SK	11	0	11	6	5	6	5	4	4	4	4	4	4	4	4	11	21
167PRECNANT, INTERIM KILL	11	0	11	6	5	6	5	4	4	4	4	4	4	4	4	11	21

DAY 4 COLUMNS - PRE- AND POSTCULLING RESPECTIVELY

NR-NOT PRECNANT PIK-PRECNANT, INTERIM KILL NR-NOT MATED SK-SCHEDULED KILL

STUDY NO. 189970 ORAL DEVELOPMENTAL REPRODUCTION STUDY WITH BUTYL BENZYL PHTHALATE IN Wistar Rats (F0-GENERATION)

APPENDIX:19 DELIVERY AND LITTER DATA GROUP D; HIGH DOSE BBP

FEMALE	LITTER DELIVERED			NUMBER OF LIVE PUPS												IMPLAN-TATIONS		DURATION OF GESTATION (DAYS)
	LIVE DEAD TOTAL			1			4			7			14			M	F	
	S	N	N	M	F	N	M	F	N	M	F	N	M	F	M			
169 SK	9	0	9	4	5	4	4	5	4	4	4	4	4	4	4	4	11	21
171 SK	10	0	10	4	6	4	4	5	4	4	4	4	4	4	4	4	11	21
173 SK	11	0	11	4	7	4	4	7	4	4	4	4	4	4	4	4	11	21
175 SK	7	0	7	6	1	6	1	6	1	6	1	6	1	6	1	6	11	22
177NOT PREGNANT																		
179 SK	11	0	11	2	9	2	9	2	9	2	6	2	6	2	6	2	11	21
181 SK	9	2	11	6	3	6	3	5	3	5	3	5	3	5	3	13	22	
183 PII	12	0	12	7	5	0	0	0	0	0	0	0	0	0	0	12	21	
185 SK	10	1	11	5	5	5	5	4	4	4	4	4	4	4	4	11	21	
187 SK	11	0	11	5	6	5	6	4	4	4	4	4	4	4	4	11	21	
189 SK	6	2	8	2	4	2	2	1	2	0	2	0	2	0	2	11	22	
191 SK	11	0	11	8	3	8	3	5	3	5	3	5	3	5	3	11	21	
193 SK	10	0	10	7	3	7	3	5	3	5	3	5	3	5	3	10	21	
195 SK	11	0	11	5	6	5	6	4	4	4	4	4	4	4	4	11	22	
197 SK	10	0	10	8	2	8	2	6	2	6	2	6	2	6	2	10	22	
199 SK	10	1	11	5	5	5	5	4	4	4	4	4	4	4	4	11	21	
201 SK	15	0	15	9	6	1	1	1	1	1	1	1	1	1	1	15	21	
203 SK	11	1	12	6	5	6	5	4	4	4	4	4	4	4	4	13	22	
205 SK	11	0	11	7	4	7	4	4	4	4	4	4	4	4	4	12	22	
207 SK	10	0	10	3	7	3	7	3	7	3	5	3	5	3	5	12	22	
209 SK	13	0	13	5	8	5	8	4	4	4	4	4	4	4	4	14	21	
211 SK	7	0	7	2	5	2	5	2	5	2	5	2	5	2	5	9	22	
213NOT PREGNANT																		
215 SK	12	0	12	9	3	9	3	5	3	5	3	5	3	5	3	12	21	
217 SK	8	0	8	5	3	5	3	5	3	5	3	5	3	5	3	9	23	
219NOT PREGNANT																		
221 SK	6	0	6	2	4	2	4	2	4	2	4	2	4	2	4	9	21	
223NOT PREGNANT																		

DAY 4 COLONIES - PRE- AND POSTCULLING RESPECTIVELY

SK-SCHEDULED KILL

PIK-PREGNANT, INTERIM KILL



16-JAN-98

Reprotox version: 2.4

Attachment III, Table 4

STUDY NO.197579 ORAL DEVELOPMENTAL REPRODUCTION STUDY WITH  
 BUTYL BENZYL PHENYLATE IN WISTAR RATS (Additional study)  
 (F0-GENERATION)

TABLE 56 NATURAL DELIVERY DATA AND LITTER DATA -- SUMMARY

	A CONTROL	C MID DOSE BBP	D HIGH DOSE BBP
Females placed with males	28	28	28
Females mated	27	28	27
Pre-coital time (days)	MEAN 2.45 u S.E. 0.273	2.61 0.243	3.00 0.287
Day 1 to 4	25 f 96	28 100	24 89
Day 5 to 7	1 f 3.0	0 0.0	3 11
Day 1 to 7	26 f 100	28 100	27 100
Females pregnant	26 f	23	26
Females with liveborns	76 f	22	24
Mating index	96	100	96
Female fecundity index	96	82	96
Female fertility index	93	82	93
Gestation index	100	96	92
Duration of gestation	MEAN 21.24 u S.E. 0.119	21.13 0.095	21.42 0.099
Females surviving delivery as % of pregnant females	26 f 100	23 100	26 100
Females with stillborn pups as % of pregnant females	5 f 19	2 8.7	4 15
Females with all stillborn pups as % of pregnant females	0 f 0.0	1 4.3	2 7.7

Statistical test: f- Fisher's exact test u- Kruskal-Wallis & Mann-Whitney U

STUDY NO.197570 ORAL DEVELOPMENTAL REPRODUCTION STUDY WITH BUTYL BENZYL PHTHALATE IN WISTAR RATS (Additional study) (F0-GENERATION)

TABLE 56 NATURAL DELIVERY DATA AND LITTER DATA -- SUMMARY

	A CONTROL		C MID DOSE BBP		D HIGH DOSE BBP	
Pups delivered (total)	N	299	248	277		
	MEAN	11.50 m	10.78	10.65		
	S.E.	0.310	0.422	0.461		
Liveborn	N	286 f	240	249*		
Live Birth Index		96	97	90		
Stillborn	N	13 f	8	27*		
Pup mortality day 1	%	4.3	3.2	9.7		
Number of pups lost (dying, missing, and/or cannibalized) on the following days:						
days 1-4	N	29 f	11*	42*		
Pup mortality day 4	%	10	4.6	17		
Called day 4	N	70	63	51		
Number alive, after culling at day 4	N	187	166	156		
Number of pups lost (dying, missing, and/or cannibalized) on the following days:						
days 5-7	N	0 f	0	0		
Pups alive day 7	N	187 f	166	156		
Viability index day 4-7	%	100	100	100		
Number of litters lost entirely (Stillborn, dying, missing, cannibalized, and/or culled) in the period between:						
days 0-4	N	1 f	2	5		
	%	3.8	9.1	21		
days 5-7	N	0 f	0	0		
	%	0.0	0.0	0.0		
days 0-7	N	1 f	2	5		
	%	3.0	9.1	21		

Statistical key: f= Fishers exact test u= Kruskal-Wallis & Mann-Whitney U \* = p<0.05

STUDY NO. 1975F0 ORAL DEVELOPMENTAL REPRODUCTION STUDY WITH BUTYL BENZYL PHTHALATE IN WISTAR RATS (Additional study) (F0-GENERATION)

TABLE 56 NATURAL DELIVERY DATA AND LITTER DATA -- SUMMARY

	A CONTROL		C MID DOSE BSP		D HIGH DOSE BSP	
Live Pups/Litter						
day 1	MEAN	11.00 v	10.91	10.38		
	S.E.	0.384	0.389	0.481		
day 4 preculling	MEAN	10.28 u	10.90	9.86		
	S.E.	0.596	0.344	0.630		
day 4 postculling	MEAN	7.48 v	7.90	7.43		
	S.E.	0.332	0.095	0.335		
day 7	MEAN	7.48 u	7.90	7.43		
	S.E.	0.332	0.095	0.335		
Number of male pups at day 1	n	154 f	123	128		
Sex ratio at day 1	g	54	51	51		
Number of male pups at day 7	n	101 f	80	76		
Sex ratio at day 7	g	54	48	49		
No of lost implantations	n	37	33	60		
Post implantations loss g	n	10.74 u	11.19	17.88		
	S.E.	2.873	4.423	5.563		

Statistical key: f= Fishers exact test u= Kruskal-Wallis & Mann-Whitney v

Mating index : number of females mated \* 100 / number of females placed with males  
 Female fecundity index : number of females pregnant \* 100 / number of females mated  
 Female fertility index : number of females pregnant \* 100 / number of females placed with males  
 Gestation index : number of females with live pups \* 100 / number of pregnant females  
 Live birth index : number of pups born alive \* 100 / total number of pups born  
 Pup mortality day n : number of dead pups on day n \* 100 / total number of pups on day n  
 Viability index day 4-7 : number of pups surviving 7 days / number of liveborn after culling at day 4  
 Sex ratio : number of male pups date n \* 100 / total number of pups  
 Post-implantation loss : number of implantation sites - number of pups born alive \* 100 / number of implantation sites

Second Mating - Data on a litter basis - *Data summarized, raw data follows.*

# of litters with at least one stillborn pup	5/26	NA	2/23	4/26
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# of litters with postnatal mortality	8/26	NA	3/23	8/26
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STUDY NO. 197578 ORAL DEVELOPMENTAL REPRODUCTION STUDY WITH BUTYL BENZYL PHTHALATE IN NISTAR RATS (Additional study) (F0-GENERATION)

APPENDIX:57 DELIVERY AND LITTER DATA GROUP A: CONTROL

FEMALES	LITTER DELIVERED			NUMBER OF LIVE PUPS							IMPLAN- TATIONS	DURATION OF GESTATION (DAYS)
	LIVE	DEAD	TOTAL	1 M	1 F	4 M	4 F	7 M	7 F			
1 SK	11	0	11	9	2	9	2	6	2	6	2	21
3 SK	10	0	10	6	4	6	4	4	4	4	4	21
5 SK	6	6	12	4	2	0	0	0	0	0	0	22
7 SK	12	0	12	4	0	4	0	4	4	4	4	22
9 SK	10	0	10	4	6	1	1	1	1	1	1	22
13 SK	9	0	9	4	5	4	5	4	4	4	4	21
13NOT PREGNANT												
15 SK	11	0	11	6	5	6	5	4	4	4	4	21
17 SK	9	3	10	4	5	4	4	4	4	4	4	22
19 SK	12	0	12	6	6	6	6	4	4	4	4	21
21 SK	10	2	12	7	3	2	0	2	0	2	0	21
23 SK	10	3	13	6	4	6	4	4	4	4	4	22
25 SK	11	0	11	6	5	6	5	4	4	4	4	22
27 SK	8	0	8	3	5	3	5	3	5	3	5	21
29 SK	10	0	10	8	2	8	2	6	2	6	2	21
31 SK	14	0	14	6	8	6	7	4	4	4	4	22
33 SK	12	0	12	6	6	6	6	4	4	4	4	21
35 SK	11	0	11	7	4	7	4	4	4	4	4	22
37 SK	11	0	11	3	8	3	7	3	5	3	5	21
39NOT MATED												
41 SK	13	0	13	8	5	8	5	4	4	4	4	no day @ pc
43 SK	14	0	14	5	9	5	8	4	4	4	4	21
45 SK	11	0	11	8	3	8	3	5	3	5	3	21
47 SK	9	1	10	5	4	5	2	5	2	5	2	21
49 SK	14	0	14	6	8	6	8	4	4	4	4	21
51 SK	12	0	12	5	7	5	7	4	4	4	4	21
53 SK	14	0	14	11	3	10	2	6	2	6	2	20
55 SK	12	0	12	7	5	7	5	4	4	4	4	20

DAY 4 COUNDS - PRE- AND POSTCULLING RESPECTIVELY

MP-NOT PREGNANT IM-NOT MATED SK-SCHEDULED KILL

STUDY NO. 1975F0 ORAL DEVELOPMENTAL REPRODUCTION STUDY WITH  
 BUTYL BENZYL PHTHALATE IN WISTAR RATS (Additional study)  
 (F0-GENERATION)

APPENDIX:57 DELIVERY AND LITTER DATA  
 GROUP C; MID DOSE B3P

FEMALE#	LITTER DELIVERED	LIVE		DEAD		TOTAL	LITTER		NUMBER OF LIVE PUPS												IMPLAN- TATIONS	DURATION OF GESTATION (DAYS)		
		M		F			M		F		1				4				7					
		N	N	N	N		M	F	M	F	M	F	M	F	M	F	M	F	M	F				
57 SK	12	0	12	0	12	6	4	0	4	4	4	4	4	4	4	4	4	4	4	12	21			
59 NOT PRESENT																								
61 SK	11	0	11	0	11	0	3	0	3	5	3	5	3	5	3	5	3	5	3	12	21			
63 SK	11	0	11	0	11	6	5	6	5	4	4	4	4	4	4	4	4	4	4	11	21			
65 SK	11	0	11	0	11	6	5	6	5	4	4	4	4	4	4	4	4	4	4	12	21			
67 SK	10	0	10	0	10	3	7	3	7	3	5	3	5	3	5	3	5	3	5	12	21			
69 SK	9	0	9	0	9	2	7	2	7	2	6	2	6	2	6	2	6	2	6	10	21			
71 SK	6	0	6	0	6	4	2	4	2	4	2	4	2	4	2	4	2	4	2	6	21			
73 NOT PRESENT																								
75 SK	12	0	12	0	12	1	11	1	11	1	7	1	7	1	7	1	7	1	7	12	22			
77 NOT PRESENT																								
79 SK	10	0	10	0	10	4	6	4	6	4	4	4	4	4	4	4	4	4	4	13	21			
81 SK	11	0	11	0	11	6	5	6	5	4	4	4	4	4	4	4	4	4	4	12	21			
83 SK	11	0	11	0	11	7	4	7	4	4	4	4	4	4	4	4	4	4	4	11	22			
85 SK	12	0	12	0	12	7	5	7	5	4	4	4	4	4	4	4	4	4	4	12	21			
87 SK	8	0	8	0	8	6	2	0	0	0	0	0	0	0	0	0	0	0	0	12	22			
89 SK	0	7	7	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	22			
91 SK	11	0	11	0	11	5	6	5	6	4	4	4	4	4	4	4	4	4	4	11	21			
93 NOT PRESENT																								
95 SK	13	0	13	0	13	6	7	6	7	4	4	4	4	4	4	4	4	4	4	14	20			
97 SK	10	0	10	0	10	6	4	6	4	4	4	4	4	4	4	4	4	4	4	12	21			
99 SK	11	0	11	0	11	5	6	5	6	5	3	5	3	5	3	5	3	5	3	12	21			
101 SK	13	0	13	0	13	9	4	0	4	4	4	4	4	4	4	4	4	4	4	13	21			
103 NOT PRESENT																								
105 SK	12	0	12	0	12	0	4	0	4	4	4	4	4	4	4	4	4	4	4	12	21			
107 SK	14	0	14	0	14	7	7	6	6	4	4	4	4	4	4	4	4	4	4	14	21			
109 SK	13	1	14	0	14	5	8	5	8	4	4	4	4	4	4	4	4	4	4	14	21			
111 SK	9	0	9	0	9	4	5	4	5	4	4	4	4	4	4	4	4	4	4	10	21			

DAY 4 COLUMNS - PRE- AND POSTCULLING RESPECTIVELY

NP-NOT PRESENT SK-SCHEDULED KILL

ReproTox version: 2.4

STUDY NO. 1975F0 ORAL DEVELOPMENTAL REPRODUCTION STUDY WITH BUTYL BENZYL PHTHALATE IN WISTAR RATS (Additional study) (F0-GENERATION)

APPENDIX:57 DELIVERY AND LITTER DATA GROUP D: HIGH DOSE BDF

FEMALE#	LITTER DELIVERED		NUMBER OF LIVE PUPS												IMPLAN- TATIONS	DURATION OF GESTATION (DAYS)
	LIVE DEAD TOTAL		1		4		4		4		4		7			
	M	F	M	F	M	F	M	F	M	F	M	F	M	F		
113 SK	9	0	9	0	3	6	3	6	3	5	3	5	3	5	9	21
115 SK	11	0	11	0	6	5	6	5	4	4	4	4	4	4	13	21
117 NOT MATED																
119 SK	12	0	12	0	6	6	6	6	4	4	4	4	4	4	13	21
121 NOT PREGNANT																
123 SK	10	0	10	0	3	7	3	7	3	5	3	5	3	5	12	21
125 SK	14	0	14	0	7	7	7	7	4	4	4	4	4	4	14	22
127 SK	1	1	13	1	8	4	8	4	4	4	4	4	4	4	13	21
129 SK	11	0	11	0	6	5	6	4	4	4	4	4	4	4	12	22
131 SK	10	0	10	0	5	5	5	5	4	4	4	4	4	4	10	22
133 SK	12	0	12	0	6	6	6	6	0	0	0	0	0	0	12	21
135 SK	14	0	14	0	7	7	7	7	4	4	4	4	4	4	14	21
137 SK	12	0	12	0	7	5	7	5	4	4	4	4	4	4	12	22
139 SK	0	11	11	0	0	0	0	0	0	0	0	0	0	0	13	22
141 SK	5	0	5	0	4	1	4	1	4	1	4	1	4	1	5	22
143 SK	10	0	10	0	1	9	1	9	1	7	1	7	1	7	11	22
145 SK	11	0	11	0	8	3	8	3	5	3	5	3	5	3	13	21
147 SK	10	0	10	0	4	6	4	6	0	0	0	0	0	0	12	22
149 SK	12	0	12	0	6	6	1	1	1	1	1	1	1	1	13	21
151 SK	12	0	12	0	6	6	6	5	4	4	4	4	4	4	12	21
153 SK	4	0	4	0	4	0	0	0	0	0	0	0	0	0	12	21
155 SK	0	9	10	0	0	0	0	0	0	0	0	0	0	0	14	22
157 SK	10	0	10	0	4	6	4	6	4	4	4	4	4	4	13	22
159 SK	11	0	11	0	6	5	6	5	4	4	4	4	4	4	12	21
161 SK	8	0	8	0	6	2	6	2	6	2	6	2	6	2	9	22
163 SK	10	0	10	0	6	4	6	4	4	4	4	4	4	4	10	21
165 SK	11	0	11	0	6	5	5	5	4	4	4	4	4	4	12	21
167 SK	6	6	14	0	3	5	3	5	1	4	1	4	1	4	14	21

DAY 4 COLUMNS - PRE- AND POSTCULLING RESPECTIVELY

BP-NOT PREGNANT    BP-NOT MATED    SK-SCHEDULED KILL

FEB - 3 1998

## TNO Nutrition and Food Research Institute

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26 January 1998

Our number

DToX/98-0130 WaI

Your letter

Dr R.S. Nair  
Solutia Inc.  
10300 Olive Boulevard  
P.O. Box 66760  
St. Louis Missouri 63166-6760  
USA

Subject

Pup mortality postnatal day 1-4

Dear Rashmi,

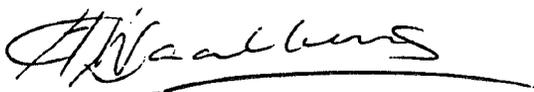
In both the initial (TNO study no. 1899) and the additional study (TNO study no. 1975) with butyl benzyl phthalate a rather unexpected high number of stillborn pups and pup mortality between postnatal day 1 and 4 was observed in the concurrent control group. In comparison to our historical data (see attachment 1) with this strain of Wistar rats this finding is not within our normal range. However, it appeared that during the year 1997 a change in the number of stillborn pups and pup mortality between postnatal day 1 and 4 (as can be seen from the table in attachment 2), is observed. A reason for this phenomenon is not yet found.

For all studies Wistar rats (CrI:(WI)WU BR) were supplied by Charles River Wiga GmbH, Sulzfeld, Germany. The animals were fed Rat and Mouse No. 3 Breeding diet, RM3, supplied by SDS Special Diets Services, Witham, England. For the studies different batches of this diet were used. At the start of the mating period the females were about 13-16 weeks of age. The housing (cages including bedding) during the gestation and lactation period were similar in all studies and the same as used in the years before. For your information, in both reference studies mentioned in attachment 2, the effect in the treatment groups did not show any clear dose response relationship for peri-and postnatal mortality. In reference study 1 a slightly higher pup mortality was observed in the low dose group but not in the mid and high dose groups. Also in both studies no treatment related effects on other reproductive parameters, maternal body weight and food consumption were observed.

Within 2-5 months data will be available from 4 other studies with the same strain of rats.

I hope this information is of help to you.

Kind regards,



Ir Ine D.H. Waalkens-Berendsen  
Product Manager Reproduction Toxicology



Department of Neurotoxicology and Reproduction Toxicology

**ATTACHMENT 1: Historical data from studies performed during the last 2 years**

Study no.	# of litters with at least 1 stillborn pup	# of litters with all stillborn pups	# of stillborn pups	# of litters lost completely postnatally	# of pups died postnatally
A	2	0	2	0	1
B	1	1	1	0	0
C	1	1	1	0	3
D	2	0	6	1	7
E	0	0	0	0	0
F	2	0	3	1	2
G	2	0	2	1	7

PM= pre-mating period, L= lactation period, w= week

**ATTACHMENT 2: Studies performed from May 1997- January 1998**

Study no.	Start PM	Start L	# of litters with at least 1 stillborn pup	# of litters with all stillborn pups	# of stillborn pups	# of litters with postnatal mortality	# of litters lost completely postnatally	# of pups died postnatally
1899	w 20 '97	w 25 '97	4	1	15	2	0	2
ref. 1	w 28 '97	w 41 '97	1	0	3	3	0	3
ref. 2	w 38 '97	w 51 '97	6	0	24	5	3	18
1975	w 48 '97	w 1 '98	5	0	13	8	1	29

PM= pre-mating period, L= lactation period, w= week