Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

International Chemical Identification:

Name:	**6,6'-di-tert-butyl-2,2'methylene-di-p-cresol, DBMC**
EC Number:	**204-327-1**
CAS Number:	**119-47-1**
Index Number:	**-**

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Version number: 1 **Date: 23 February 2018**

Note on confidential information

Please be aware that this report is intended to be made publicly available. Therefore it should not contain any confidential information. Such information should be provided in a separate confidential Annex to this report, clearly marked as such.

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1 PHYSICAL HAZARDS

Not assessed.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

No studies available.

3 HEALTH HAZARDS

Acute toxicity

Not assessed.

3.1 Acute toxicity - oral route

Not assessed.

3.2 Acute toxicity - dermal route

Not assessed.

3.3 Acute toxicity - inhalation route

Not assessed.

3.4 Skin corrosion/irritation

Not assessed.

3.5 Serious eye damage/eye irritation

Not assessed.

3.6 Respiratory sensitisation

Not assessed.

3.7 Skin sensitisation

Not assessed.

3.8 Germ cell mutagenicity

Not assessed.

3.9 Carcinogenicity

Not assessed.

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 Study 1: Reproduction toxicity screening test

Study reference:

Publication type: Summary report

Authors: Ministry of Health and Welfare, Japan.

Title of the report: Toxicity testing report of environmental chemicals, volume 7. p. 423-437. Preliminary Reproduction Toxicity Screening Test of 2,2'-methylenebis(6-tert-betyl-p-cresol) by oral Administration

Year: 1999

NB: The report is in Japanese with English abstract and summarised data and result tables in English and figures with English legends.

Detailed study summary and results:

Test type

• *Guideline:*

OECD TG. Similar to 421(the TG number is not stated in the report itself, but in OECD SIDS, 2001).

• *GLP compliant:*

Yes

Test substance

• *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:*

CAS number identical to that of substance in the CLH dossier

• *EC number:*

Not stated in the abstract or figures (the rest of the report in Japanese)

• *CAS number:*

119-47-1

• *Degree of purity:*

98.2%

• *Impurities (or a note that the impurities do not affect the classification):*

n.a.

• *Batch number:*

710140 (Lot No.) (OECD SIDS, 2001)

Test animals

• *Species/strain/sex:*

Male and female rats; Crj:CD(SD)

- *No. of animals per sex per dose:*

n=12/sex/dose

- *Age and weight at the study initiation:*

10 week old (both sexes), 206-238 g (females), 332-383 g (males) (OECD SIDS, 2001)

Administration/exposure

- *Route of administration:*

Oral (gavage)

- *Duration and frequency of test/exposure period:*

Daily administration for 50-52 days (males) and 40-48 days (females), from 14 days before mating to the day 3 of lactation

- *Doses/concentration levels:*

0, 12.5, 50, 200, 800 mg/kg bw/day test substance in 5% gum Arabic

- *Rationale for dose level selection:*

n.a.

- *Control group and treatment:*

n=12/sex control rats, concurrent vehicle (5 % gum Arabic)

- *Historical control data if available?*

n.a.

- *Vehicle:*

5 % gum Arabic

- *Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:*

n.a.

- *Actual doses (mg/kg bw/day):*

0, 12.5, 50, 200, 800 mg/kg bw/day for both sexes

- *Substance concentration (ppm) to the actual dose, if applicable:*

n.a.

Description of test design:

- *Details on mating procedure (M/F ratios per cage, length of cohabitation, proof of pregnancy):*

Male/female per cage: 1/1

Length of cohabitation: at the most 14 days, until proof of pregnancy

Proof of pregnancy: formation of vaginal closing or sperm detection in vagina

(OECD SIDS, 2001)

- *Premating exposure period for males and females:*

14 days

- *Dosing schedules and pre and post dosing observation periods:*

Daily administration of 0 (vehicle), 12.5, 50, 200, 800 mg/kg bw/day test substance for 50-52 days (Males) from 14 days prior to mating, and for 40-48 days (famales) from 14 days prior to mating to day 3 of lactation. No pre- or post-dosing observations

• *Standardization of litters (yes/no):*

n.a.

• *Parameters assessed for P animals:*

Body weight (twice a week form experimental day 0), food/water consumption (twice a week form experimental day 0), Organ weight: testis, epididymis, cauda epididymis, ovary (examined at necropsy), Sperm examination of adult male rats: motility, viability, morphology, Microscopic examinations: testis, caput epididymis (control & all treated groups); seminal vesicle, ovary (control & 800 mg/kg bw/day group), No. of pairs with successful copulation, copulation index (No. of pairs with successful copulation/No. of pairs mated x 100), pairing days until copulation, No. of pregnant females, fertility index (No. of pregnant animals/No. of pairs with successful copulation x 100), No. of corpora lutea, No. of implantation sites, implantation index (No. of implantation sites/No. of corpora lutea x 100), No. of living pregnant females, No. of pregnant females with parturition, gestation length, No. of pregnant females with live pups on day 0, gestation index (No. of females with live pups/No. of living pregnant females x 100), No. of pregnant females with live pups on day 4, delivery index (No. of pups born/No. of implantation sites x 100) (OECD SIDS, 2001)

• *Estrous cycle length and pattern, sperm examination:*

No informarion on estrous cycle length and patterns, but numbers of estrous cases (cycles) before mating (14 days) were assessed. Sperm were examined on the following parameters: motility, viability, morphology, microscopic examinations: testis, caput epididymis (control & all treated groups) (OECD SIDS, 2001).

• *Parameters assessed for F1 (pups):*

Body weight of pups: on PND 0 and 4, Microscopic examinations: full macroscopic examinations of all of pups, No. of pups alive on day 0 of lactation, live birth index (No. of live pups on day 0/No. of pups born x 100), sex ratio (Total No. of male pups/Total No. of female pups), No. of pups alive on day 4 of lactation, viability index (No. of live pups on day 4/No. of live pups on day 0 x 100), body weight of live pups (on day 0 and 4). (OECD SIDS, 2001)

• *Clinical observations performed and frequency:*

General appearance (adult rats: twice a day, pups: once a day after birth) (OECD SIDS, 2001)

• *Post exposure observation period:*

None

Results and discussion

Describe the relevant findings.

• *Actual dose received by dose level by sex if known*

0, 12.5, 50, 200, 800 mg/kg bw/day for both sexes

• *Statistical treatment of results:*

Dunnett's or Scheffe's test for continuous data and Chi square test for quantal data (OECD SIDS, 2001)

• *Provide data on any dose-related observations:*

NOAEL: Male: 12.5 mg/kg bw/day, Female: 50 mg/kg bw/day

LOAEL: Male: 50 mg/kg bw/day, Female: 200 mg/kg bw/day

12.5 mg/kg bw/day: No adverse effects in males and females

50 mg/kg bw/day:

Males: No effect on body weight or food consumption was seen during the study. At necropsy no effect on testes and epididymis weights were recorded, but giant cell formation in the testes (in 2 out of 12, slight grade) was present, however not significantly different from the control group. Furthermore, statistically significant adverse effects on sperm quality were seen, including a 16% decrease in sperm motility ratio, a 10 % decrease in sperm viability ratio, a 20% decrease in sperm survivability ratio and a ~30 % decrease in number of sperm in the epididymis cauda. Also a 5 times increase in the abnormal sperm ratio was observed.

Females: No adverse effects, including no decrease in body or ovary weight or any adverse effects on fertility were seen.

Offspring:. No significant effects.

200 mg/kg bw/day:

Males: No effect on body weight or food consumption was seen during the dosing period. At necropsy no significant effects on body weight were seen, but absolute and relative testes weights were reduced by ~ 16% and absolute and relative epididymides weights were reduced by ~ 12 %. Histopatholotological effects on the testes, including atrophy of seminiferous tubules (in 6 out of 12, slight to marked grade), and giant cell formation in the testes (in 2 out of 12, slight grade) was seen. However the incidence of giant cell formation was not significantly different form control. Furthermore histological examination of the epididymis showed a signficant increase in the number of animals with decreased sperm (in 9 out of 12, slight to marked grade). Other adverse effects on sperm included an increase in abnormal sperm ratio(from 1,55% in control to 56.3 % in this dose group), and a ~80 % decrease in the sperm motility ratio, a 26 % decrease in sperm viability ratio, ~50 % decrease in sperm survivability ratio, and ~66 % decrease in number of sperm in the epididymis cauda.

Females: during the dosing period a smaller body weight gain (non-significant) was seen compared to controls. This effect became significant in the lactation period (day1-4). Additionally, lower food consumption was seen periodically during pre-mating, pregnancy and lactation. At necropsy on PND 4, body weight was significantly decrease (by 7%), whereas absolute and relative ovary weights were not significantly affected, and neither were the number of corpora lutea and implantation scars .

Offspring: A slight decrease in number of live pups born and live pups on day 4 of lactation (~12 %) was seen, and both male and female offspring had higher birth weights than control pups, possibly due to the smaller litter size.

800 mg/kg bw/day:

Males: During the dosing period, no effect on body weight was seen, but transient decrease in food consumption (one day in the beginning of the dosing period) was observed. At necropsy no effect on body weight was noted, but absolute and relative testes weights were decreased by ~ 50 %, and a ~25% decrease was seen in absolute and relative epididymis weights. Histological examination showed severe effects on the testes, including atrophy of seminiferous tubules (in 12 out of 12, mild to marked grade), and a severe decrease in sperm was seen in the epididymis (in 12 out of 12, marked grade). . No motile sperm was evident in any of the 12 examined males, and the number of abnormal sperm was very high. Moreover, the total number of sperm in cauda epididymis was ~80 % decreased, compared to control males.

Females: A significant 14 % decrease in the number of corpora lutea and a significant 8 % decrease in number of implantation scars were seen, indicating adverse effects on sexual function and fertility. This dose caused suppression of body weight gain during pregnancy and lactation periods, and lower food consumption periodically during pre-mating, pregnancy and lactation. At necropsy body weight was 9% decreased, whereas ovary weights were not affected.

Offspring: A significant (9.6%) decrease in number of pups born.. Furthermore 1 dam was unable to deliver pups, and 1 dam lost all pups during lactation. Offspring birth weights were unaffected but a ~10 % decrease in body weight was seen in offspring of both sexes on PND 4, but this was only statistically significant in male pups.

For P animals (per dose):

• *Number of animals at the start of the test and matings:*

n=24/dose

• *Time of death during the study and whether animals survived to termination:*

All survived, until they were sacrificed at the end of the study.

• *Body weight data for P animals selected for mating:*

In male rats, there was no significant effect on body weight between any of the test groups and the control group, however a slight non-significant lower in body weight (4.6%) was seen for the 800 mg/kg bw/day group at termination (see fig. 1 and table 1).

Female rats had slightly lower body weight in the 200 mg/kg bw/day group at the end of pregnancy and the effect reached significance through the lactation periods; in the 800 mg/kg bw/day-group body weight was slightly reduced through pregnancy period and decrease further trough pregnancy and lactation (see fig. 3 and table 4).

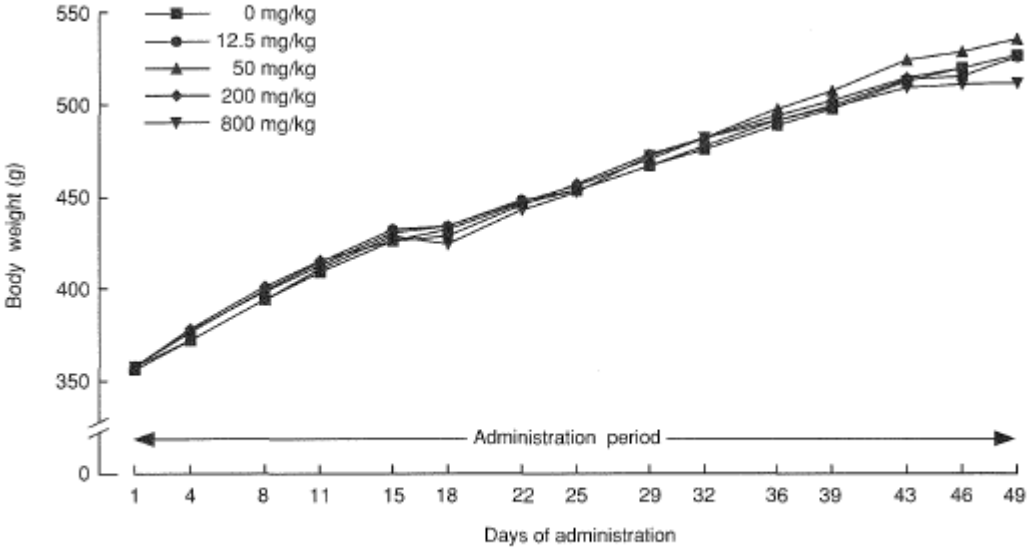


Fig. 1 Body weight of male rats in preliminary reproduction toxicity screening test of 2,2'-methylenebis(6-*tert*-butyl-*p*-cresol) by oral administration

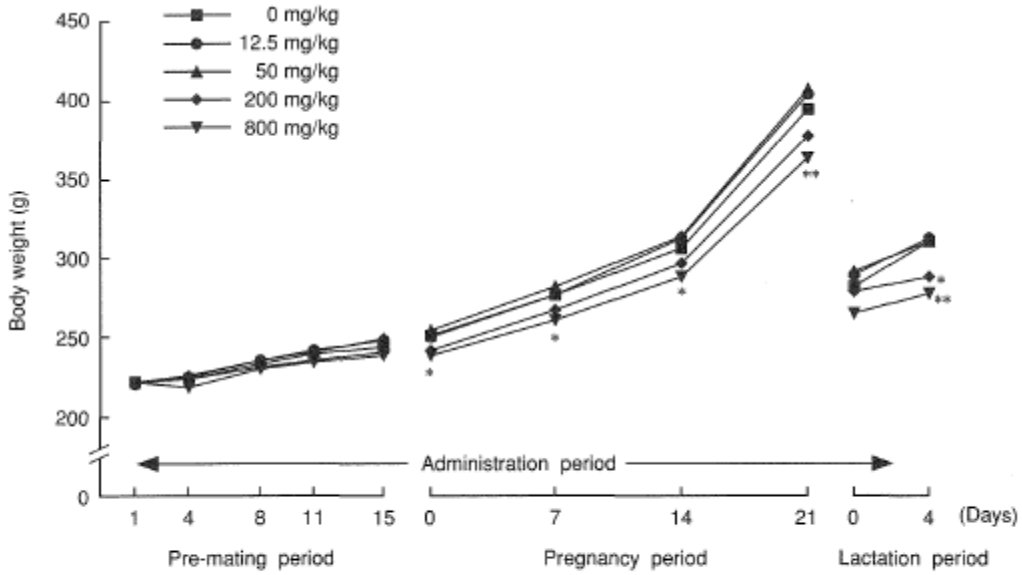


Fig. 3 Body weight of female rats in preliminary reproduction toxicity screening test of 2,2'-methylenebis(6-*tert*-butyl-*p*-cresol) by oral administration

Significantly different from control (*:p<0.05, **:p<0.01)

• *Body weight at sacrifice and absolute and relative organ weight data for the parental animals:*

No effect on body weight or organ weight at sacrifice in the 12,5 or 50 mg/kg bw/day groups.

200 mg/kg bw/day:

Males: No effect on body weight, but absolute and relative testes weights were reduced by ~ 16%, and absolute and relative epididymides weights were reduced by ~ 12 %. (see table 1).

Females: Body weight was significantly lower (7%) than in controls at study termination. No effect on reproductive organ weights. (see table 4).

800 mg/kg bw/day:

Males: A slight non-significant lower in body weight (4.6%) was reported at study termination. Absolute and relative testes weights were decreased by ~ 50 %, and a ~25% decrease was seen in absolute and relative epididymis weight. (see table 1).

Females: Body weight was significantly decreased by 9% at study termination. No effect on reproductive organs weight was seen. (see table 4).

Table 1 Organ weight of male rats in preliminary reproduction toxicity screening test of 2,2'-methylenebis (6-*tert*-butyl-*p*-cresol) by oral administration

Dose (mg/kg)		0	12.5	50	200	800
Number of males		12	12	12	12	12
Body weight	(g)	535.8 ± 41.7	536.5 ± 33.5	544.3 ± 31.4	539.7 ± 35.7	514.0 ± 34.4
Testes	(g)	3.550 ± 0.333	3.598 ± 0.320	3.558 ± 0.302	2.983 ± 0.767*	1.736 ± 0.263**
	(g%)	0.666 ± 0.082	0.674 ± 0.072	0.655 ± 0.046	0.557 ± 0.157*	0.338 ± 0.050**
Epididymides	(g)	1.255 ± 0.143	1.343 ± 0.118	1.196 ± 0.113	1.108 ± 0.125*	0.924 ± 0.100**
	(g%)	0.235 ± 0.034	0.250 ± 0.024	0.220 ± 0.018	0.205 ± 0.027*	0.180 ± 0.020**

Each value shows mean±S.D.
Significantly different from control (*: $p<0.05$, **: $p<0.01$).

Table 4 Organ weight of female rats in preliminary reproduction toxicity screening test of 2,2'-methylenebis (6-*tert*-butyl-*p*-cresol) by oral administration

Dose (mg/kg)		0	12.5	50	200	800
Number of dams		12	12	12	12	12
Body weight	(g)	310.4 ± 12.3	312.2 ± 18.9	310.7 ± 17.2	287.4 ± 13.3**	281.9 ± 22.9**
Ovaries	(mg)	94.50 ± 12.06	91.43 ± 10.00	89.88 ± 8.77	89.69 ± 16.74	88.78 ± 14.65
	(mg%)	30.51 ± 4.20	29.36 ± 3.40	28.90 ± 1.90	31.23 ± 5.75	31.44 ± 3.93

Each value shows mean ± S.D.
Significantly different from control (**: $p<0.01$).

• *Other organ weight changes if available:*

No data available

• *Toxic response data by sex and dose including indices of mating, fertility, gestation, birth, viability and lactation; indicate the numbers used in calculating the indices:*

No significant effects on number of estrous cases before mating, copulation index, number of concerving days, fertility index or number of pregnant females with live pups (although 1 out of 12 dams (in the 200 mg/kg bw/day group) and 2 out of 12 dams (in the 800 mg/kg bw/day group) were not pregnant with live pups, where for the rest of the groups 12 out of 12 dams were pregnant with live pups) (see table 6 below for gestation, birth and viability). No information on lactation

Table 5 Number of estrous cases and reproductive performance of male and female rats in preliminary reproduction toxicity screening test of 2,2'-methylenebis(6-*tert*-butyl-*p*-cresol) by oral administration

Dose (mg/kg)	0	12.5	50	200	800
Number of females	12	12	12	12	12
Number of estrous cases before mating (14 days)					
Mean±S.D.	3.3 ± 0.5	3.5 ± 0.5	3.5 ± 0.5	3.7 ± 0.5	3.2 ± 0.6
Number of pairs	12	12	12	12	12
Number of pairs with successful copulation	12	12	12	12	12
Copulation index (%)[a]	100.0	100.0	100.0	100.0	100.0
Number of conceiving days (Mean±S.D.)	2.3 ± 1.3	2.3 ± 1.1	2.6 ± 1.2	3.1 ± 1.8	1.7 ± 1.2
Number of pregnant females	12	12	12	11	12
Fertility index (%)[b]	100.0	100.0	100.0	91.7	100.0
Number of pregnant females with live pups	12	12	12	11	10

a) : (Number of pairs with successful copulation/number of pairs) × 100.
b) : (Number of pregnant females/number of pairs with successful copulation) × 100.

• *Clinical observations:*

Non were stated

• *Haematological and clinical biochemistry findings:*

No data available

• *Data on functional observations:*

n.a.

• *Necropsy findings:*

n.a.

Histopathological findings: nature and severity: See table 3 below

50 mg/kg bw/day:

Slight grade of giant cell formation in the testes (in 2 out of 12) was present, but not significantly different from control.

200 mg/kg bw/day:

Histopatholotological effects on the testes, including atrophy of seminiferous tubules (in 6 out of 12, slight to marked grade) and giant cell formation in the testes (in 2 out of 12, slight grade) were seen. However the incidence of giant cell formation was not significantly different form control. Furthermore a decrease in sperm was seen in the epididymis (in 9 out of 12, slight to marked grade).

800 mg/kg bw/day:

Histopatholotological effects on the testes, including atrophy of seminiferous tubules (in 12 out of 12, mild to marked grade), and a severe decrease in sperm was seen in the epididymis (in 12 out of 12, marked grade).

Table 3 Histopathological examination of male rats in preliminary reproduction toxicity screening test of 2,2'-methylenebis(6-*tert*-butyl-*p*-cresol) by oral administration

Dose (mg/kg)	0						12.5						50						200						800					
Incidence & grade	N	A	±	+	2+	3+	N	A	±	+	2+	3+	N	A	±	+	2+	3+	N	A	±	+	2+	3+	N	A	±	+	2+	3+
Testis	[12]						[12]						[12]						[12]						[12]					
Atrophy, seminiferous tubule	12	0					12	0					12	0					6	6	1	2	2	1**	0	12	0	1	10	1**##
Degenelation, seminiferous tubule	12	0					12	0					12	0					11	1	1	0	0	0	12	0				
Decrease, sperm	12	0					12	0					12	0					11	1	1	0	0	0	12	0				
Giant cell formation	12	0					12	0					10	2	2	0	0	0	10	2	2	0	0	0	12	0				
Epididymis	[12]						[12]						[12]						[12]						[12]					
Decrease, sperm	12	0					12	0					12	0					3	9	2	2	1	4**	0	12	0	0	0	12**##

Grade of histopathological finding; ±:slight, +:mild, 2+:moderate, 3+:marked.
N:No abnormality detected.
A:Abnormality detected.
[ ]:Number of males examined.
Significantly different from control (**:p<0.01).
Significantly different by dose response test (##:p<0.01).

- *Effects on sperm:* (See table 2 below)

50 mg/kg bw/day:

Statistically significant adverse effects on sperm quality were seen, including a 16% decrease in sperm motility ratio, a 10 % decrease in sperm viability ratio, a 20% decrease in sperm survivability ratio and a ~30 % decrease in number of sperm in the epididymis cauda. Also a 5 times increase in the abnormal sperm ratio was observed.

200 mg/kg bw/day:

The adverse effects on sperm were very severe and included an increase in abnormal sperm ratio(from 1,55% in control to 56.3 % in this dose group), and a ~80 % decrease in the sperm motility ratio, a 26 % decrease in sperm viability ratio, ~50 % decrease in sperm survivability ratio, and ~66 % decrease in number of sperm in the epididymis cauda.

800 mg/kg bw/day:

No motile sperm was evident in any of the 12 examined males, and the number of abnormal sperm was very high. Moreover, the total number of sperm in cauda epididymis was ~80 % decreased, compared to control males.

Table 2 Observation of sperm in preliminary reproduction toxicity screening test of 2,2'-methylenebis (6-*tert*-butyl-*p*-cresol) by oral administration

Dose (mg/kg)	0	12.5	50	200	800
Number of males	12	12	12	12	12
Sperm motion parameters					
After 30 min. incubation					
Motility ratio (%)	71.96 ± 9.69	74.92 ± 7.81	60.42 ± 10.26**	14.50 ± 21.75**	0.00 ± 0.00**
Path velocity (μm/s)	149.45 ± 10.72	157.08 ± 6.58	155.81 ± 9.56	128.07 ± 40.54 (6)	-
Straight line velocity (μm/s)	100.28 ± 13.08	108.18 ± 6.58	105.81 ± 11.07	83.88 ± 27.65 (6)	-
Curvilinear velocity (μm/s)	348.95 ± 20.87	369.08 ± 16.17*	364.94 ± 18.14	301.08 ± 104.59 (6)	-
Beat cross frequency (Hz)	30.64 ± 1.77	30.16 ± 1.59	32.91 ± 1.70**	29.98 ± 10.51* (6)	-
Morphology of sperm					
Abnormal ratio (%)[a]	1.55 ± 3.63	0.55 ± 0.55	8.11 ± 6.33**	56.33 ± 29.03** (7)	91.70 (1)
Viability (%)[b]	98.57 ± 2.04	99.56 ± 0.47	89.19 ± 11.47**	71.68 ± 9.31** (4)	-
Survivability (%)[c]	83.29 ± 6.87	86.44 ± 3.27	66.03 ± 17.79**	39.03 ± 15.16** (4)	-
Number of sperms in left epididymis cauda ($\times 10^6$)	207.41 ± 60.16	222.42 ± 49.26	128.00 ± 39.88**	60.73 ± 29.17**	30.30 ± 15.98**
Number of sperms/g (left epididymis cauda) ($\times 10^6$)	707.85 ± 153.02	704.85 ± 154.64	503.29 ± 159.44**	238.88 ± 102.42**	138.92 ± 83.31**

Each value shows mean±S.D.
Significantly different from control (*: $p<0.05$, **: $p<0.01$).
Figures in parenthses indicate number of males.
a) : (Number of abnormal sperms/300 sperms) × 100.
b) : ((Number of live sperms + number of died sperms during the 2 hrs. incubation) /300 sperms) × 100.
c) : (Number of live sperms/300 sperms) × 100.

Effects on pregnancies and pups: see table 6 below:

• *Number of P females cycling normally and cycle length:*

n.a.

• *Precoital interval (number of days until mating and number of estrous periods until mating):*

n.a.

• *Duration of gestation (calculated from day 0 of pregnancy):*

No significant effect on length of gestation (days), or gastation index (number of dams with live pups/number of pregnant dams)/100))*).*

• *Number of implantations, corpora lutea, litter size:*

200 mg/kg bw/day*:*

No effect on the number of corpora lutea, implantation scars or implantation index ((number of implantation scars/ number of corpora lutea)x100). Significant decrease in number of pups born by ~ 13 %, a ~12 % decrease in live pups born and a ~7% decrease in delivery index (number of pups born/number of implantation scars)x100))

800 mg/kg bw/day:

A ~14 % significant decrease in the number of corpora lutea, ~ 8 % significant decrease in number of implantation scars, but no significant effect on implantation index or delivery index. Significant decrease in number of pups born by 9.6 %

- *Number of pre- and post-implantation loss:*
- n.a. *Number of dams with abortions, early deliveries, stillbirths, resorptions and/or dead foetuses:*

No effect on the numbers of dead pups on day 0 of lactation.

The number of dams with abortions, early deliveries, stillbirths or resorptions were not stated in the abstract or figures (the rest of the report in Japanese).

- *Number of live births:*

No effect on live birth index (number of live pups born/number of pups born)x100)), but significant decrease by ~12 % in number of live pups born in the 200 mg/kg bw/day dose group

For F1 pups/litters (per dose):

- *Mean number of live pups (litter size)*

A slight, but significant decrease by ~12 % in number of live pups born in the 200 mg/kg bw/day dose group

- *Sex ratio:*

No effect on sex ratio at birth

- *Viability index (pups surviving 4 days/total births):*

Number of live pups on day 4 were significantly decreased by ~12 % in the 200 mg/kg bw/day group, but no effect on viability index ((number of live pups on day 4/number of live pups born)x100)

- *Survival index at weaning*

Not stated in the abstract or figures

- *Mean litter or pup weight by sex:*

There is a significant increase in mean male and female pup weight at postnatal day 0 in the 200 mg/kg bw/day dose groups and a significant decrease in mean male pup weight at postnatal day 4 in the 800 mg/kg bw/day dose groups

- *External, soft tissue and skeletal malformations and other relevant alterations:*

No external malformations or alterations

- *Number and percent of fetuses and litters with malformations (including runts) and/or variations as well as description and incidences of malformations and main variations (and/or retardations)*

No external anormalities. Soft tissue and skeletal malformations were not assessed

Table 6 Observation of pups in preliminary reproduction toxicity screening test of 2,2'-methylenebis (6-*tert*-butyl-*p*-cresol) by oral administration

Dose (mg/kg)	0	12.5	50	200	800
Number of dams	12	12	12	12	12
Length of gestation (days)	22.08 ± 0.29	22.00 ± 0.00	22.33 ± 0.49	22.45 ± 0.52	22.27 ± 0.65
Corpora lutea	16.4 ± 3.0	16.4 ± 2.6	16.3 ± 1.5	15.1 ± 1.4	14.1 ± 1.6*
Implantation scars	14.3 ± 3.0	14.7 ± 1.1	15.2 ± 1.3	13.5 ± 1.4	13.1 ± 1.5*
Implantation index (%)[a]	86.9 ± 14.7	91.0 ± 12.8	93.6 ± 7.4	90.1 ± 8.5	93.1 ± 7.2
Gestation index (%)[b]	100.0	100.0	100.0	100.0	83.3
Pups born	13.5 ± 3.3	13.5 ± 1.0	14.8 ± 1.3	11.7 ± 1.4**	12.2 ± 1.8*
Delivery index (%)[c]	93.5 ± 8.9	92.2 ± 5.2	97.3 ± 3.3	87.2 ± 10.5*	92.8 ± 5.7
Live pups born	13.1 ± 3.2	13.3 ± 0.8	14.3 ± 1.3	11.5 ± 1.0**	11.3 ± 4.1
Sex ratio at birth[d]	0.70 ± 0.45	1.02 ± 0.37	1.43 ± 0.92	1.03 ± 0.70	1.25 ± 0.68
Birth index (%)[e]	90.7 ± 9.4	91.2 ± 4.8	94.7 ± 4.4	86.0 ± 9.6	83.8 ± 28.8
Dead pups on day 0 of lactation	0.4 ± 0.7	0.2 ± 0.4	0.4 ± 0.5	0.2 ± 0.6	0.9 ± 2.7
Live birth index (%)[f]	97.1 ± 4.6	98.9 ± 2.5	97.2 ± 3.5	98.8 ± 3.9	89.9 ± 30.0
Live pups on day 4 of lactation	13.1 ± 3.2	13.3 ± 0.8	14.3 ± 1.4	11.4 ± 1.0**	12.4 ± 1.8 (10)
Viability index (%)[g]	100.0 ± 0.0	100.0 ± 0.0	99.4 ± 2.0	98.5 ± 3.4	100.0 ± 0.0 (10)
External anomalies (%)[h]	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0 (10)
Body weight of pups (g)					
Male Day 0	6.78 ± 0.40	6.81 ± 0.23	6.90 ± 0.50	7.60 ± 0.52**	6.97 ± 0.71 (10)
Male Day 4	11.02 ± 0.83	11.05 ± 0.77	10.58 ± 1.11	11.29 ± 1.14	9.68 ± 1.72* (10)
Female Day 0	6.43 ± 0.37	6.40 ± 0.17	6.53 ± 0.44	7.25 ± 0.49**	6.61 ± 0.66 (10)
Female Day 4	10.33 ± 0.81	10.36 ± 0.67	10.20 ± 1.15	10.77 ± 1.04	9.30 ± 1.64 (10)

Each value shows mean±S.D. per dam.
Figures in parentheses indicate number of dams.
Significantly different from control (*: $p<0.05$, **: $p<0.01$).
a) : (Number of implantation scars/number of corpora lutea) × 100.
b) : (Number of dams with live pups/number of pregnant dams) × 100.
c) : (Number of pups born/number of implantation scars) × 100.
d) : Number of male pups/number of female pups.
e) : (Number of live pups born/number of implantation scars) × 100.
f) : (Number of live pups born/number of pups born) × 100.
g) : (Number of live pups on day 4/number of live pups born) × 100.
h) : (Number of pups with external anomalies/number of live pups born) × 100.

- *Data on physical landmarks in pups and other postnatal developmental data:*

n.a.

- *Data on functional observations*

n.a.

3.10.1.2 Study 2: Subchronic toxicity study

Study reference:

Publication type: Peer reviewed scientific article.

Authors: Atsuya Takagi, Koichi Takada, Kimic Sai, Toshiaki Ochiai, Kiyoshi Matsumoto, Kiyoshi Sekita, Junko Momma, Yoshitaka Aida, Minoru Saitoh, Katsushi Naitoh, Tsuyoshi Furuya, Ryuichi Hasegawa and Yuji Kurokawa

Title of the article: Acute, Subchronic and Chronic toxicity studies of a synthetic antioxidant 2,2'methylenebis(4-methyl-6-tert-butylphenol) in rats

Journal: The journal of Toxicological Sciences, Vol. 19, 77-88

Year: 1994

Remark: The summary below describes the subchronic toxicity part of the study. The chronic part of the study is described below as study 3.

Test type

• *Guideline:*

Not stated

GLP compliant:

No

Test substance

• *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:*

It has the same CAS number as the substance in the CLH dossier.

• *EC number:*

Not stated

• *CAS number:*

119-47-1

• *Degree of purity:*

Not stated

• *Impurities (or a note that the impurities do not affect the classification):*

Not stated

• *Batch number:*

Not stated

Test animals

• *Species/strain/sex:*

Male rats; wistar

• *No. of animals per sex per dose:*

n=5/sex/dose for both 4 and 12 week exposure periods (in total n=10/sex/dose)

• *Age and weight at the study initiation:*

5 weeks old (both sexes), ca 115 g (females), ca 140 g (males) (read from growth curve)

Administration/exposure

• *Route of administration:*

Oral (feed)

• *Duration and frequency of test/exposure period:*

Daily administration (in diet) for 4 and 12 weeks

• *Doses/concentration levels, rationale for dose level selection:*

0, 1200, 6000, 30.000 ppm (0, 88, 564, 3120 mg/kg bw/day (males) and 0, 104, 618, 2610 mg/kg bw/day (females)), no rationale for dose level selection stated.

• *Post exposure observation period*

None

• *Control group and treatment:*

n=5/sex control rats for each exposure period (4 and 12 weeks), recived normal diet without the test substance

• *Vehicle:*

Normal feed without the test substance

• *Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:*

The basal diet powder was purchased from Funabashi Farm and the test substance was mixed into the diet prior to pelleting at the desired concentrations and stored at 4ºC until use.

• *Actual dose (mg/kg bw) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable*

0, 1200, 6000, 30.000 ppm (0, 88, 564, 3120 mg/kg bw/day (males) and 0, 104, 618, 2610 mg/kg bw/day (females))

• *Statistical methods*

All quantitative data except for the histopathological findings were statistically analyzed by one-way analysis of variance (ANOVA) techniques with Dunnett's or Scheffe's multiple comparison procedures. Histopathological data were statistically analyzed with the cumulative Chi-squares test

Description of test design:

• *Parameters assessed:*

Body weight (measured weekly), Mean food intake, survival rate, weight of organs, histological assesments (of testis, epididymis, seminal vesicle, prostate, ovary uterus, thymus, bone marrow and haemorrhage)

Blood was collected from the orbital venous plexus under ether anesthesia after 4 and 12 weeks of exposure to the test substance, and the hematological parameters, like red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean hemoglobin concentration (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelets (PLT) and white blood cells (WBC) were assessed.

Serum biochemical assessments: total protein (T-PRO), albumin (ALB), blood urea nitrogen (BUN), creatinine (CRN), uric acid, (UA), glucose(GLU), non-esterified fatty acids (NEFA), phospholipids (PL), triglycerides (T-GLY), total cholesterol (T-CHO), free cholesterol (F-CHO), alkaline phosphatase (ALP), amylase (AMY), cholinesterase (CHE), aspartate aminotransferase (AST), alanine aminotransferase (ALT), Y-glutamyl trans peptidase (Y-GTP), 2-hydroxybutyrate dehydrogenase (HBDH), leucine aminopeptidase (LAP) and lactate dehydrogenase (LDH) and calcium (Ca), inorganic phosphorus (Pi), sodium (Na), potassium (K), chloride (Cl) and magnesium (Mg) Fresh urine was examined for pH, protein, glucose, ketone bodies, occult blood, bilirubin and urobilin in the morning at weeks 4 and 12.

At autopsy, the weights of the brain, heart, lungs, liver, kidneys, spleen, adrenal, testes, ovaries, pituitary and thyroid glands were measured. The above-mentioned organs and the salivary glands, esophagus, stomach, small and large intestine, pancreas, urinary bladder, seminal vesicles, epididymis, ischiatic nerve, uterus, prostate, mesenteric lymph nodes, thymus, spinal cord, skeletal muscle and bone marrow (femur and sternum) were fixed in 10% buffered formalin solution for routine histological processing. Paraffin sections were stained with hematoxylin and eosin for histopathological examination.

• *Estrous cycle length and pattern, sperm examination:*

Estrous were not investigated and testis were investigated by fixing them in 10% buffered formalin solution and staining with hematoxylin and eosin for histopathological examination.

Clinical observations performed and frequency:

General appearance (daily monitored)

Results and discussion

Describe the relevant findings.

• *Provide data on any dose-related observations:*

No NOAEL was found.

LOAEL of 1200 ppm (88 mg/kg bw/day, female; 104 mg/kg bw/day, male)

Males: Body weight gain was not significantly affected during the study, and was ~4% decreased when measures at the 12 week necropsy. Absolute liver weight was not significantly affected, but relative liver weight was 24 % increased (after 12 weeks). Reproductive function was affected, seen as a non-significant (9%) decrease in absolute testis weight after 4 weeks, and a significant ~50% decrease after 12 weeks. Decreased spermatogenisis (after 4 and 12 weeks), seen as testicular tubule atrophy (in 5 out of 5 after 12 weeks), appearance of giant cells (in 3 out of 5 at 4 weeks), interstitial edema in the testis (in 4 out of 5 at

12 weeks), sermatogenic arrest in testes (in 5 out of 5 at both 4 and 12 weeks) and hypospermia in the epididymis (in 5 out of 5 at 4 and 12 weeks).

Females: Body weight was not affected during the study, but a non-significant ~9 % decreased was seen at 12 weeks compared to controls. Absolute and relative liver weights were not significantly affected, but relative liver weights were non-significantly increased by ~14% after 4 weeks and ~22% increased after 12 weeks. After 4 weeks absolute and relative ovary weight were significantly decreased (~24 %), whereas the 9 % decrease in absolute ovary weight seen after 12 weeks was not statistically significant. No histopathological findings in uterus and ovary. In the haematological and biochemical findings a significant decrease of hemoglobin (HGB) was observed at week 12.

6000 ppm (564 mg/kg bw/day, female; 618 mg/kg bw/day, male)

Males: increased mortality (survival rate referred as 45% in table; however, does appear to be 100% after 4 weeks and 60% after 12 weeks) probably due to haemorrhage, decreased food intake, reduced body weight (~ 44 % after 4 and 12 weeks), increased relative liver weights after 4 and 12 weeks, thymus atrophy and bone marrow hypoplasia noted. Decrease in absolute testis weight (after 4 and 12 weeks) and decrease in relative testis (after 12 weeks) decrease of spermatogenisis (after 4 weeks and hereafter), testicular atrophy and interstitial edema in the testis (at 12 weeks), at week 4 and 12, atrophy of the seminal vesicles and prostate. In the haematological and biochemical findigs was observed a significant decrease in the red blood cells (RBC) (at week 12), hemoglobin (HGB) (at week 12), cholinesterase (CHE) activity (at week 4), phospholipids (PL) (at week 4), total colesterol (T-CHO) and free colesterol (F-CHO) (after 4 and 12 weeks) and a significant increase of γ-glutamyl transpeptidase (γ-GTP) (at week 12). Note that n=2 in regard to data generated at 12 weeks.

Females: decreased body weight (27 % after 4 weeks and 37 % after 12 weeks), increase in absolute and relative liver weights (after 4 and 12 weeks), atrophy of the ovaries and uterus, histopathological changes in ovaries and uterus, and a decrease in absolute ovary weights (after 4 and 12 weeks). In the haematological and biochemical findigs was observed a significant decrease of hemoglobin (HGB) (at week 12), glucose (GLU) (at week 4) and cholinesterase (CHE) activity (at week 4 and 12). Significant increase of phospholipids (PL) (at week 4 and 12), total colesterol (T-CHO) and free colesterol (F-CHO) (at week 4 and 12) and γ-glutamyl transpeptidase (γ-GTP) (at week 4 and 12).

30000 ppm (3120 mg/kg bw/day, female; 2610 mg/kg bw/day, male)

Males: increased mortality (survival rate 20% after 12 weeks - 80% after 4 weeks) decreased food intake, reduced body weight (half the size of controls after 4 weeks and 1/3 the size after 12 weeks, note n=1), increased relative liver weights by ~ 89% at 4 weeks, thymus atrophy and bone marrow hypoplasia, diarrhoea, biochemical changes in blood but not urine, decrease in absolute (by ~ 69%, after 4 weeks) and relative (by ~ 83%, after 4 weeks) testis weight, decrease of spermatogenisis (after 4 weeks and hereafter), testicular atrophy and interstitial edema in the testis (at 12 weeks), at week 4 and 12, epididymis atrophy, hypospermia and atrophy of the seminal vesicles and prostate. In the haematological and biochemical

findigs was observed a significant decrease of hemoglobin (HGB) (at week 4), glucose (GLU) (at week 4), cholinesterase (CHE) activity (at week 4). Although not significant, a decrease of platlets (PLT) was noted at week 12. Significant increase of phospholipids (PL) (at week 4), and total colesterol (T-CHO) and free colesterol (F-CHO) were significantly elevated at week 4 and non-significantly at week 12 (note n=1 at 12 weeks).Note that n=1 in regard to data generated at 12 weeks.

Females: increased mortality (survival rate reported as 36% appears to be 80% after 4 uger, 40% after 12 weeks) decreased food intake. increased relative liver weights (by ~ 148% after 4 weeks and ~ 185% after 12 weeks) and absolute liver weights (by ~ 51% after 4 weeks and ~ 59% after 12 weeks) and a decrease in absolute ovary weights (by ~ 56% after 4 weeks and ~ 40% after 12 weeks) and relative ovary weights (by ~ 30% after 4 weeks and ~ 32% after 12 weeks)... Histopathological changes including: Atrophy of the ovaries, uterus and thymus were noted. Furtermore bone marrow hypoplasia was observed , . In the haematological and biochemical findigs was observed a significant decrease in hemoglobin (HGB) (at week 12), glucose (GLU) (at week 4 and 12), cholinesterase (CHE) activity (at week 4 and 12). Although not significant, a decrease of platlets (PLT) was noted (at week 12). Significant increase of phospholipids (PL) (at week 12), total colesterol (T-CHO) (at 4 and 12 weeks), free colesterol (F-CHO) (at week 12 and non-significantly at week 4) and γ-glutamyl transpeptidase (γ-GTP) (at week 4 and 12). Note that n=2 in regard to data generated at 12 weeks

None of the testes doses affected weights of brain, heart, lung, kidney, spleen, adrenals, pituitary or thyroids for any of the doses

- *body weight and body weight changes*

Severe suppression of body weight gain was observed in the 0.6% and 3.0% groups (both sexes) (see fig 2 below)

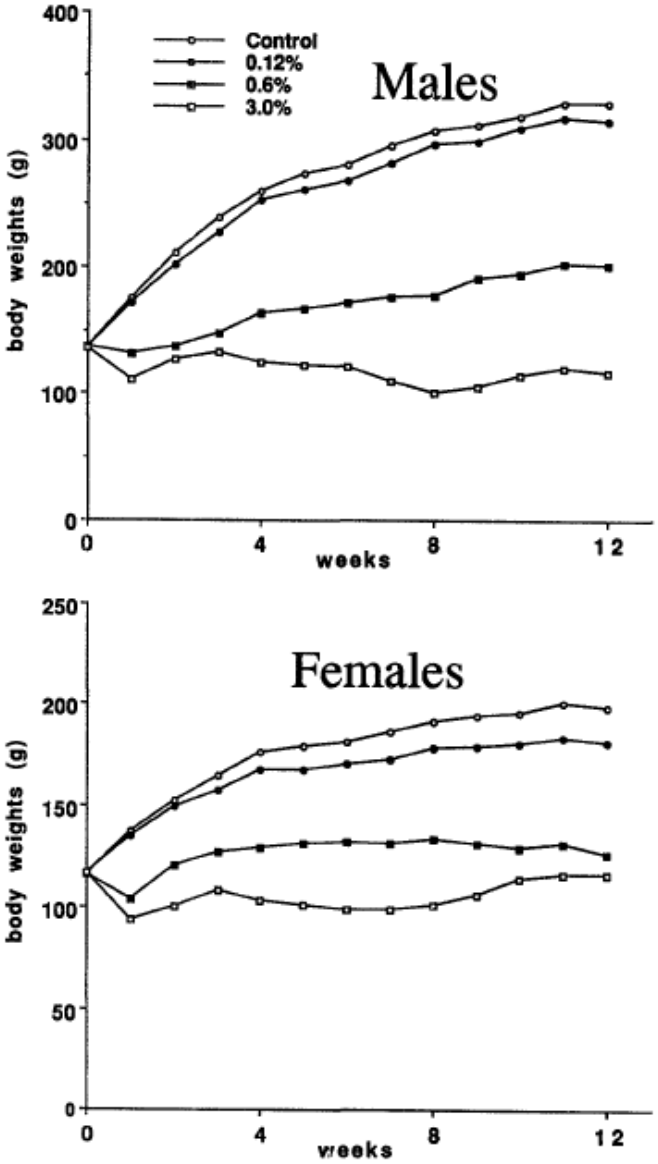


Fig. 2. Growth curves for rats fed MBMBP for 12 weeks.

• *Body weight at sacrifice and absolute and relative organ weight data for the parental animals:*

Significantly decrease in terminal body weight in the 0.6% and 3.0% groups for both sexes after 4 and 12 weeks of exposure to test substance

1200 ppm (88 mg/kg bw/day, female; 104 mg/kg bw/day, male)

Male: Absolute liver weight was not significantly affected, but relative liver weight was 24% increased (after 12 weeks). A non-significant (9%) decrease in absolute testis weight after 4 weeks, and a significant ~50% decrease in absolute and relative testis weight after 12 weeks

Female: Body weight was decreased non-significantly by ~9 % at 12 weeks compared to controls. Absolute and relative liver weights were not significantly affected, but relative liver weights were non-significantly increased ~14% after 4 weeks and ~22% after 12 weeks . After 4 weeks absolute and relative ovary weight

were significantly decreased (~24 %), whereas the 9 % decrease in absolute ovary weight seen after 12 weeks was not statistically significant

6000 ppm (564 mg/kg bw/day, female; 618 mg/kg bw/day, male)

Male: Increased relative liver weights after 4 and 12 weeks. Decrease in absolute testis weight (after 4 and 12 weeks) and decrease in relative testis (after 12 weeks). Note that n=2 in regard to data generated at 12 weeks.

Female: Increase in absolute and relative liver weights (after 4 and 12 weeks) and a decrease in absolute ovary weights (after 4 and 12 weeks)

30000 ppm (3120 mg/kg bw/day, female; 2610 mg/kg bw/day, male)

Males: Reduced body weight (half the size of controls after 4 weeks and 1/3 the size after 12 weeks, note n=1). Increased relative liver weights by ~ 89% at 4 weeks and decrease in absolute (by ~ 69%, after 4 weeks) and relative (by ~ 83%, after 4 weeks) testis weight. Note that n=1 in regard to data generated at 12 weeks.

Females: increased relative liver weights (by ~ 148% after 4 weeks and ~ 185% after 12 weeks) and absolute liver weights (by ~ 51% after 4 weeks and ~ 59% after 12 weeks) and a decrease in absolute ovary weights (by ~ 56% after 4 weeks and ~ 40% after 12 weeks) and relative ovary weights (by ~ 30% after 4 weeks and ~ 32% after 12 weeks). Note that n=2 in regard to data generated at 12 weeks.

(See table 3)

Table 3. Body and organ weights for rats fed diet containing MBMBP for up to 12 weeks.

Weeks	Groups	B.W. (g)	N[a]	Absolute organ weights		Relative organ weights	
				Liver (g)	Testis (g) or ovary (mg)	Liver (g%)	Testis (g%) or ovary (mg%)
Males							
4	0	232±6	5	7.39±0.32	2.89±0.17	3.19±0.12	1.25±0.09
	0.12	222±12	5	8.34±0.40	2.62±0.16	3.76±0.11	1.18±0.09
	0.6	129±16**	5	6.91±0.97	1.26±0.32**	5.34±0.30*	0.99±0.31
	3.0	111±6**	4	6.75±1.69	0.90±0.25**	6.03±1.17**	0.81±0.21*
12	0	314±18	4	8.12±0.57	2.99±0.10	2.58±0.05	0.95±0.06
	0.12	300±14	5	9.61±0.67	1.45±0.11**	3.20±0.10**	0.48±0.03**
	0.6	178±29**	2	7.95±0.72	0.84±0.10**	4.49±0.31**	0.47±0.02**
	3.0	110	1	6.86	0.78	6.23	0.71
Females							
4	0	162±8	5	4.65±0.37	83±7	2.88±0.12	52±4
	0.12	154±3	5	5.08±0.43	62±6*	3.29±0.33	40±4*
	0.6	117±16**	5	6.50±1.23*	57±11**	5.54±0.46**	49±7
	3.0	99±12**	4	7.03±1.08**	36±9**	7.13±0.72**	36±5**
12	0	188±12	5	4.40±0.19	66±11	2.34±0.16	37±5
	0.12	170±9	5	4.85±0.29	60±2	2.86±0.12	35±2
	0.6	118±29**	5	6.21±1.56*	34±11**	5.29±0.54*	29±4
	3.0	105±5**	2	7.01±0.77*	26±3**	6.71±1.08*	25±1**

a : Number of rats tested. Values are the means±S.D. for data from all rats.
Significantly different from control ; *P<0.05, **P<0.01.

• *Clinical observations: description, severity, time of onset and duration.*

Severe suppression of body weight gain was observed in the 0.6% and 3.0 % groups. Decreases of mean food intake per rat were observed for the 0,6 and 3.0% males and for the 3,0% female. Deaths were observed in the 0.6% and 3.0% males and 3.0% females from 3 weeks to 4, all mortalities being accompanied by hemorrhage fron nasal cavity. Diarrhea was ovserved in two of four of the 3.0 % males and in one of the four 3.0% females which died. Since all mortalities showed hemorrhage from the nasal cavity, the cause of death was concluded to be hemorrhage.

• *Sensory activity, grip strength and motor activity assessments (when available)*

No data available

• *Ophthalmologic findings: incidence and severity*

No data available

• *Gross pathology findings: incidence and severity*: not reported.

• *Histopathology findings: incidence and severity:*

See table 4 below for an overview of the histological findings after 4 and 12 weeks exposure in thymus, testis, epididymis, seminal vesicle, prostate and bone marrow (male rats); and in thymus, ovary, uterus and bone marrow (female rats)

1200 ppm (88 mg/kg bw/day, female; 104 mg/kg bw/day, male)

Males: Decreased spermatogenesis (after 4 and 12 weeks), seen as testicular tubule atrophy after 12 weeks (in 5 out of 5, severe), appearance of giant cells at 4 weeks (in 3 out of 5, slight/mild), interstitial edema at 12 weeks (in 4 out of 5, mild), sermatogenic arrest after 4 weeks (in 5 out of 5, sligt/mild) and after 12 weeks (in 5 out of 5, severe) and hypospermia in the epididymis at 4 weeks (in 5 out of 5, mild/marked) and at 12 weeks (in 5 out of 5, severe).

Females: No histopathological findings in uterus and ovary or thymus.

6000 ppm (564 mg/kg bw/day, female; 618 mg/kg bw/day, male)

Males: Decrease of spermatogenisis (after 4 weeks and hereafter), seen as testicular tubule atrophy at 4 weeks (in 5 out of 5, slight/mild) and at 12 weeks (in 2 out of 2, marked/severe), appearance of giant cells at 4 weeks (in 5 out of 5, mild/marked/severe) and at 12 weeks (in 1 out of 2, mild), spermatogenic arrest at 4 weeks (in 5 out of 5, mild) and at 12 weeks (in 2 out of 2, severe), interstitial edema at 12 weeks (in 2 out of 2, mild), , hypospermia at 4 weeks (in 5 out of 5, severe) and at 12 weeks (in 2 out of 2, severe), atrophy of the seminal vesicles at 4 weeks (in 2 out of 5, mild) and at 12 weeks (in 1 out of 2, slight) and atrophy of the prostate at 4 weeks (in 5 out of 5, slight/mild) and at 12 weeks (in 1 out of 2, slight). Atrophy of the thymus at 4 weeks (in 4 out of 5, slight) and at 12 weeks (in 2 out of 2, slight). Bone marrow hypoplasia was observed at 4 weeks (in 5 out of 5, slight/mild) and at 12 weeks (in 1 out of 2, slight)

Females: Atrophy of the ovaries at 4 and 12 weeks (in 3 out of 5, slight) and of the uterus at 4 and 12 weeks (in 2 out of 5, slight), atrophy of the thymus at 4 weeks (in 1 out of 5, slight) and at 12 weeks (in 3 out of 5, slight) Bone marrow hypoplasia was observed at 4 weeks (in 3 out of 5, slight) and at 12 weeks (in 3 out of 5, slight/mild).

30000 ppm (3120 mg/kg bw/day, female; 2610 mg/kg bw/day, male)

Males: decrease of spermatogenisis (after 4 weeks and hereafter), seen as testicular tubule atrophy at 4 weeks (in 4 out of 4, slight/mild) and at 12 weeks (in 1 out of 1, mild), appearance of giant cells at 4 weeks (in 4 out of 4, marked/severe), spermatogenic arrest at 4 weeks (in 4 out of 4, mild) and at 12 weeks (in 1 out of 1, severe) , interstitial edema in the testis at 12 weeks (in 1 out of 1, mild), epididymis atrophy at 4 weeks (in 3 out of 4, slight/mild) and at 12 weeks (in 1 out of 1, mild), hypospermia at 4 weeks (in 4 out of 4, severe) and at 12 weeks (in 1 out of 1, severe), atrophy of the seminal vesicles at 4 weeks (in 3 out of 5, slight/mild) and at 12 weeks (in 1 out of 1, mild) and atrophy of the prostate at 4 weeks (in 4 out of 4, mild) and at 12 weeks (in 1 out of 1, slight). Atrophy of the thymus at 4 weeks (in 4 out of 4, mild) and at 12 weeks (in 1 out of 1, mild). Bone marrow hypoplasia was observed at 4 weeks (in 3 out of 4, slight/marked) and at 12 weeks (in 1 out of 1, marked).

Females: Atrophy of the ovaries at 4 (in 3 out of 4, slight) and at 12 weeks (in 2 out of 2, slight). Atrophy of the uterus at 4 weeks (in 4 out of 4, slight) and at 12 weeks (in 2 out of 2, slight). atrophy of the thymus at 4 weeks (in 2 out of 4, slight/mild) and at 12 weeks (in 2 out of 2,mild). Bone marrow hypoplasia was observed at 4 weeks (in 2 out of 4, slight) and at 12 weeks (in 1 out of 2, slight). Hemorrhage was observed in 1 out of 2 after 12 weeks.

Table 4. Histological findings for rats fed diet containing MBMBP for up to 12 weeks.

Findings	Group	4 weeks 0%	4 weeks 0.12%	4 weeks 0.6%	4 weeks 3.0%	12 weeks 0%	12 weeks 0.12%	12 weeks 0.6%	12 weeks 3.0%
Males		(5)	(5)	(5)	(4)	(4)	(5)	(2)	(1)
Thymus									
atrophy	±	0	0	4	0	0	0	2	0
	+	0	0	0	4	0	0	0	1
Testis									
tubules									
atrophy	±	0	0	4	2	0	0	0	0
	+	0	0	1	2	0	0	0	1
	++	0	0	0	0	0	0	1	0
	+++	0	0	0	0	0	5	1	0
appearance of giant cells									
	±	0	2	0	0	0	0	0	0
	+	0	1	2	0	0	0	1	0
	++	0	0	2	2	0	0	0	0
	+++	0	0	1	2	0	0	0	0
spermatogenic arrest									
	±	0	2	0	0	0	0	0	0
	+	0	3	5	4	0	0	0	0
	++	0	0	0	0	0	0	0	0
	+++	0	0	0	0	0	5	2	1
calcification									
	±	0	0	0	0	0	1	0	0
	+	0	0	0	0	0	1	0	0
	++	0	0	1	0	0	0	1	0
interstitial edema									
	+	0	0	0	0	0	4	2	1
Epididymis									
atrophy	±	0	0	0	1	0	0	0	0
	+	0	0	0	2	0	0	0	1
hypospermia									
	+	0	2	0	0	0	0	0	0
	++	0	3	0	0	0	0	0	0
	+++	0	0	5	4	0	5	2	1
Seminal vesicle									
atrophy	±	0	0	0	1	0	0	1	0
	+	0	0	2	2	0	0	0	1
Prostate									
atrophy	±	0	0	3	0	0	0	1	1
	+	0	0	2	4	0	0	0	0
Bone marrow									
hypoplasia	±	0	0	2	2	0	0	1	0
	+	0	0	3	0	0	0	0	0
	++	0	0	0	1	0	0	0	1
hemorrhage									
	+	0	0	0	0	0	0	0	0
Females		(5)	(5)	(5)	(4)	(5)	(5)	(5)	(2)
Thymus									
atrophy	±	0	0	1	1	0	0	3	0
	+	0	0	0	1	0	0	0	2
Ovary									
atrophy	±	0	0	3	3	0	0	3	2
Uterus									
atrophy	±	0	0	2	4	0	0	2	2
Bone marrow									
hypoplasia	±	0	0	3	2	0	0	2	1
	+	0	0	0	0	0	0	1	0
	++	0	0	0	0	0	0	0	0
hemorrhage									
	+	0	0	0	0	0	0	0	1

( ) : No. of animals examined histologically. ± : slight, + : mild, ++ : marked, +++ severe.

- *Haematological and clinical biochemistry findings if available:*

For the hematological and serum biochemical analyses, only parameters demonstrating significant changes in either sex are listed in table 2. There were no changes in the biochemical parameters for urine from treated rats (data not shown).

Table 2. Hematological and serum biochemical findings for rats fed diet containing MBMBP for up to 12 weeks.

Weeks	Group	N[a]	RBC ($10^6/\mu l$)	HGB (g/dl)	PLT ($10^3/\mu l$)	GLU (mg/dl)	PL (mg/dl)	T-CHO (mg/dl)	F-CHO (mg/dl)	CHE (mU/ml)	γ-GTP (mU/ml)
Males	0	5	8.30 ±0.39	15.7 ±0.3	983 ±136	121 ±4	149 ±16	61 ±14	38 ±8	651 ±129	0.0 ±0.0
4	0.12	5	7.57 ±0.45	14.3 ±0.7	1065 ±127	124 ±3	152 ±9	70 ±5	42 ±5	517 ±35	0.0 ±0.0
	0.6	5	7.62 ±0.51	14.5 ±1.1	1014 ±365	112 ±16	191* ±25	109** ±26	68** ±16	427* ±85	0.0 ±0.0
	3.0	4	7.23 ±0.62	13.5* ±1.0	1280 ±459	94** ±12	206** ±17	144** ±20	68* ±14	429* ±87	3.4 ±3.2
12	0	4	9.25 ±0.32	15.6 ±0.5	746 ±15	116 ±8	158 ±11	75 ±14	46 ±7	765 ±63	0.0 ±0.0
	0.12	5	9.24 ±0.22	15.4 ±0.3	794 ±33	121 ±13	186 ±20	105* ±16	59 ±11	803 ±199	0.0 ±0.0
	0.6	3	8.45* ±0.18	14.0** ±0.3	818 ±38	92 ±31	192 ±29	113* ±10	70* ±5	932 ±748	1.6** ±0.9
	3.0	1	8.47	14.2	573	93	235	219	137	485	16
Females	0	5	7.80 ±0.22	15.4 ±0.3	744 ±90	115 ±10	170 ±6	105 ±5	48 ±2	1486 ±153	0.1 ±0.2
4	0.12	5	7.43 ±0.98	14.5 ±1.9	786 ±120	109 ±9	180 ±8	138 ±15	54 ±8	1259 ±244	0.5 ±0.5
	0.6	5	7.55 ±1.09	15.2 ±0.9	1144 ±256	99* ±12	224** ±25	216* ±41	75* ±7	845** ±44	6.0** ±2.5
	3.0	4	7.32 ±0.26	14.2 ±0.4	784 ±136	95** ±6	221 ±48	217* ±75	60 ±19	649** ±221	12.7** ±4.7
12	0	5	8.60 ±0.28	16.0 ±0.6	704 ±48	107 ±11	171 ±8	111 ±13	35 ±3	1800 ±258	0.3 ±0.4
	0.12	5	8.13 ±0.24	15.0* ±0.4	753 ±48	114 ±11	181 ±16	149 ±29	42 ±9	1686 ±342	1.3 ±0.2
	0.6	5	8.43 ±0.21	14.8** ±0.5	767 ±160	93 ±14	229** ±12	209** ±52	61** ±14	840** ±189	10.4* ±1.8
	3.0	2	8.88 ±0.12	13.9** ±0.2	372 ±22	72* ±5	265** ±22	286** ±24	80** ±1	728** ±326	18.4* ±1.4

a : Number of rats tested. Values are the means ± S.D. for data from all rats.
Significantly different from control ; *P<0.05, **P<0.01.

Significant decrease in the red blood cells (RBC) was observed in 0.6% males at week 12, but not in treated females. Significant decrease of hemoglobin (HGB) was observed in 3.0% males at week 4, and 0.6% males and all treated females at week 12. Although not significant, a decrease of platlets (PLT) in both sexes of the 0.3% group noted at week 12. Significant decrease of glucose (GLU) was observed in 3.0% males and 0.6% females at week 4 and in 3.0% females at week 4 and week 12. At week 4 phospholipids

(PL) was significantly increased in 0.6% and 3.0% males and 0.6% females and at week 12 in 0.6% and 3.0% females. Total colesterol (T-CHO) and free colesterol (F-CHO) were significantly elevated in both sexes of the 0.6 and 3.0% groups at week 4 and thereafter, except for 3.0% females at week 4 and 3.0% males at week 12 (note n=1). Cholinesterase (CHE) activity was significantly decreased in both males and females receiving 0.6 or 3.0% at week 4 and in 0.6 and 3.0% females at week 12. γ-glutamyl transpeptidase (γ-GTP) was significantly increased in the 0.6 and 3.0% females from 4 weeks and hereafter and in the 0.6% males at week 12.

• *Food/water consumption*

The severe descrease in body weight gain observed in the two high dose groups (0.6% and 3.0%), were accompanied by a decrease in food intake when looking at g/rat/day. However when looking at g/kg BW/day the food indtake increases compared to controls. (see table 1 below for food comsumption data)

• *Mortality and time to death (if occurring)*

Deaths were observed in males in the 0.6% and 3.0% groups from weeks 3 and in females in the 3.0% group from week 4. From figure 3 below it appears that 8 out of 10 males of the high dose group died and 5 males at 0.6% died. For the females, a total of 7 rats died, all from the 3.0%. Survival rates stated in the study table 1 seem slightly erroneous at 0.6% for males and at 3.0% for females.

Table 1. Survival rate, mean food intake and mean chemical intake for rats fed MBMBP for 12 weeks.

MBMBP in the diet (%)	Males				Females			
	0	0.12	0.6	3.0	0	0.12	0.6	3.0
Survival rate at wk 12 (%)	100	100	45	20	100	100	100	36
Mean food intake (g/rat/day)	19.2	18.7	15.5	12.1	13.3	14.3	13.0	9.1
Mean food intake (g/kg BW/day)	72	73	94	104	76	87	103	87
Mean MBMBP intake (mg/kg BW/day)	0	88	564	3120	0	104	618	2610

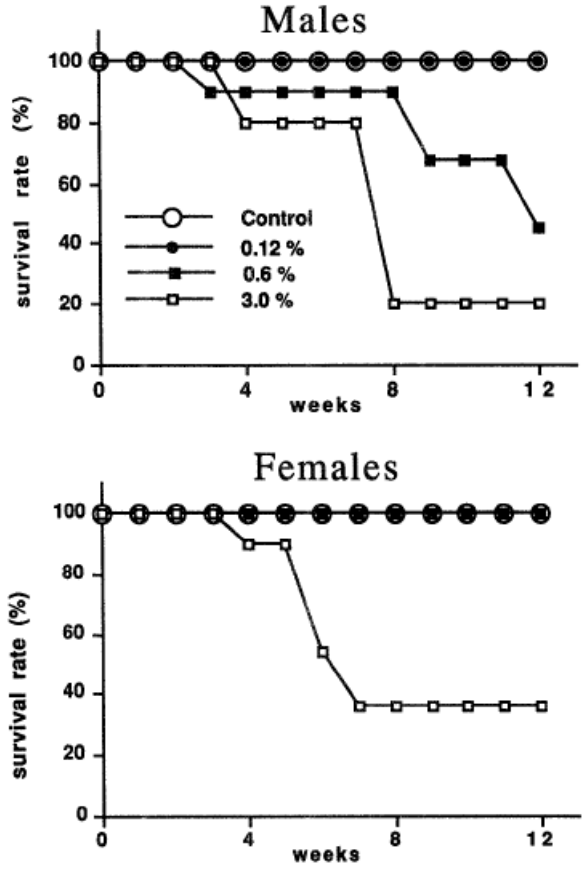


Fig. 3. Survival curves for rats fed MBMBP for 12 weeks.

• *Necropsy findings:*

Deaths were observed in both sexes, especially in males. Authors states that all mortalities showed hemorrhage from the nasal cavity, and thus concludes hemorrhage to be the cause of death.

3.10.1.3 Study 3: Chronic toxicity study

Study reference:

Publication type: Peer reviewed scientific article.

Authors: Atsuya Takagi, Koichi Takada, Kimic Sai, Toshiaki Ochiai, Kiyoshi Matsumoto, Kiyoshi Sekita, Junko Momma, Yoshitaka Aida, Minoru Saitoh, Katsushi Naitoh, Tsuyoshi Furuya, Ryuichi Hasegawa and Yuji Kurokawa

Title of the article: Acute, Subchronic and Chronic toxicity studies of a synthetic antioxidant 2,2'methylenebis(4-methyl-6-tert-butylphenol) in rats

Journal: The journal of Toxicological Sciences, Vol. 19, 77-88

Year: 1994

Remark: The summary below describes the chronic toxicity part of the study. The subchronic part of the study is described as study 2 above.

Test type

• *Guideline:*

Not stated

• *GLP compliant:*

No

Test substance

• *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:*

It has the same CAS number as the substance in the CLH dossier.

• *EC number:*

Not stated

• *CAS number:*

119-47-1

• *Degree of purity:*

Not stated

• *Impurities (or a note that the impurities do not affect the classification):*

Not stated

• *Batch number:*

Not stated

Test animals

• *Species/strain/sex:*

Male rats; Wistar

• *No. of animals per sex per dose:*

In total n=30/sex/dose, however the number of animals varies depending on which parameter that are measured. N=20/sex/dose for the histolocigal findings, n=10/sex/dose (18 month exposure period) for body and organ weight measurements and n=5/sex/dose (6 and 12 month exposure period) for body and organ weight measurements and for haematological and serum biochemical examinations.

• *Age and weight at the study initiation:*

5 weeks old (both sexes), ca 115 g(females), ca 140 g (males) (read from growth curve)

Administration/exposure

• *Route of administration:*

Oral (feed)

• *Duration and frequency of test/exposure period:*

daily administration (in diet) for 6, 12 and 18 months

• *Doses/concentration levels, rationale for dose level selection:*

0, 100, 300, 1000 ppm (0, 4, 12.7, 42.3 mg/kg bw/day (males) and 0, 5, 15.1, 54.2 mg/kg bw/day (females)), no rationale for dose level selection stated.

• *Post exposure observation period*

None

• *Control group and treatment:*

n=5-20/sex depending on the treatment and the length of test periods. Control rats, recived normal diet without the test substance

• *Vehicle:*

None. Normal feed without the test substance

• *Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:*

The basal diet powder was purchased from Funabashi Farm and the test substance was mixed into the diet prior to pelleting at the desired concentrations and stored at 4ºC until use.

• *Actual dose (mg/kg bw) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable*

0, 100, 300, 1000 ppm (0, 4, 12.7, 42.3 mg/kg bw/day (males) and 0, 5, 15.1, 54.2

• *Statistical methods*

All quantitative data except for the histopathological findings were statistically analyzed by one-way analysis of variance (ANOVA) techniques with Dunnett's or Scheffe's multiple comparison procedures. Histopathological data were statistically analyzed with the cumulative Chi-squares test

Description of test design:

• *Parameters assessed:*

Body weight (measured monthly), Mean food intake, survival rate, weight of organs, histological assesments (of testis, epididymis, seminal vesicle, prostate, ovary uterus, thymus, bone marrow and haemorrhage)

Blood was collected from the orbital venous plexus under ether anesthesia after 4 and 12 weeks of exposure to the test substance, and the hematological parameters, like red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean hemoglobin concentration (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelets (PLT) and white blood cells (WBC) were assessed Serum biochemical assessments: total protein (T-PRO), albumin (ALB), blood urea nitrogen (BUN), creatinine (CRN), uric acid, (UA), glucose(GLU), non-esterified fatty acids (NEFA), phospholipids (PL), triglycerides (T-GLY), total cholesterol (T-CHO), free cholesterol (F-CHO), alkaline phosphatase (ALP), amylase (AMY), cholinesterase (CHE), aspartate aminotransferase (AST), alanine aminotransferase (ALT), Y-glutamyl trans peptidase (Y-GTP), 2-hydroxybutyrate dehydrogenase (HBDH), leucine aminopeptidase (LAP) and lactate dehydrogenase (LDH) and calcium (Ca), inorganic phosphorus (Pi), sodium (Na), potassium (K), chloride (Cl) and magnesium (Mg) Fresh urine was examined for pH, protein, glucose, ketone bodies, occult blood, bilirubin and urobilin in the morning at weeks 4 and 12.

At autopsy, the weights of the brain, heart, lungs, liver, kidneys, spleen, adrenal, testes, ovaries, pituitary and thyroid glands were measured. The above-mentioned organs and the salivary glands, esophagus, stomach, small and large intestine, pancreas, urinary bladder, seminal vesicles, epididymis, ischiatic nerve, uterus, prostate, mesenteric lymph nodes, thymus, spinal cord, skeletal muscle and bone marrow (femur and sternum) were fixed in 10% buffered formalin solution for routine histological processing. Paraffin sections were stained with hematoxylin and eosin for histopathological examination.

- *Estrous cycle length and pattern, sperm examination:*

Estrous were not investigated and testis were investigated histopathologically, by fixing them in 10% buffered formalin solution and staining with hematoxylin and eosin.

- *Clinical observations performed and frequency:*

General appearance (daily monitored). Body weight and food onsumption were recorded monthly.

Results and discussion

Describe the relevant findings.

- *Provide data on any dose-related observations:*

100 ppm (4.23 mg/kg bw/day, male; 5.1 mg/kg bw/day, female)

Male & Female: no adverse effects

NOAEL of 300 ppm (12.7 mg/kg bw/day, male; 15.1 mg/kg bw/day, female)

Males: Body weight was not affected at any time point during the study, but was non-significantly decreased by 6% after 18 months. Absolute liver weights were not significantly increased at 6, 12 or 18

months, but relative liver weights showed a significant 9% increase at 18 months. Relative and absolute testes weights, as well as testes and epididymis histopathology, was not affected.

Females: no adverse effect were noted.

LOAEL of 1000 ppm (42.3 mg/kg bw/day, male; 54.2 mg/kg bw/day, female)

Males: Absolute and relative testes weights were significantly decreased throughout the study (by 58-75%). At all three time points, severe testis tubules atrophy, spermatogenic arrest and epididymis hypospermia was seen in all investigated animals (5/5 at 6 and 12 months and 19/19 at 18 months).. Survival rates were unaffected, small suppression of body weight gain from the 6th month but no significant body weight change at 6 and 12 months. At 18 months a significant 9% decrease in body weight was observed. Increased absolute (15-20 %) and relative (22-27%) liver weight were seen at all three time points.

Females: suppression of body weight gain from 1st months, resulting in a 23-28% decreased body weight at 12 and 18 months. Increased relative but not absolute liver weights were observed at 12 and 18 months (32-34%), whereas no significant changes were noted in the ovaries.

• *Body weight and body weight changes*

Significant suppression of body weight gain was observed in the 1.0% group (males) form month 6 and the 1.0% group (females) from month 1(see fig 4)

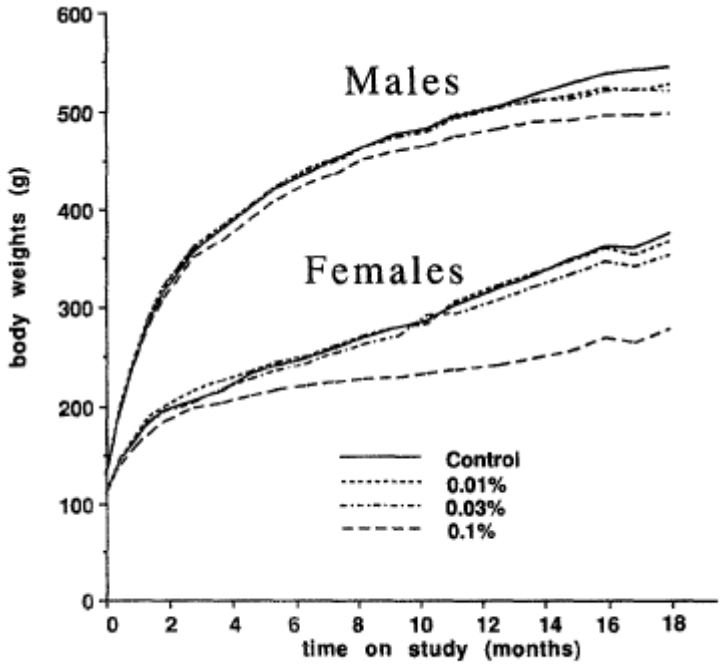


Fig. 4. Growth curves for rats fed MBMBP for 18 months.

• *Body weight at sacrifice and absolute and relative organ weight data for the parental animals:*

100 ppm (4.23 mg/kg bw/day, male; 5.1 mg/kg bw/day, female)

Male & Female: no adverse effects. See table 6 below

300 ppm (12.7 mg/kg bw/day, male; 15.1 mg/kg bw/day, female)

Males: Body weight was not significantly affected. Relative liver weights showed a significant 9% increase at 18 months. Relative and absolute testes weights was not affected.

Females: no adverse effect were noted.

1000 ppm (42.3 mg/kg bw/day, male; 54.2 mg/kg bw/day, female)

Males: Absolute and relative testes weights were significantly decreased throughout the study (by 58-75%). Small suppression of body weight gain from the 6th month but no significant body weigtht changes at 6 and 12 months. At 18 months a significant 9% decrease in body weight was observed. Increased absolute (15-20 %) and relative (22-27%) liver weight were seen.

Females: suppression of body weight gain from 1st months, resulting in a 23-28% decreased body weight at 12 and 18 months. Increased relative but not absolute liver weights were observed at 12 and 18 months (32-34%), whereas no significant changes were noted at ovaries weight.

Table 6. Body and organ weights for rats fed diet containing MBMBP for up to 18 months.

Months	Group	B.W. (g)	N[a]	Absolute organ weights		Relative organ weights	
				Liver (g)	Testis (g) or ovary (mg)	Liver (g%)	Testis (g%) or ovary (mg%)
Males							
6	0	393±21	5	9.95±0.99	3.08±0.21	2.53±0.16	0.78±0.05
	0.01	395±10	5	10.12±0.55	3.09±0.14	2.56±0.15	0.78±0.04
	0.03	396±10	5	10.73±0.29	3.01±0.11	2.71±0.06	0.76±0.02
	0.1	385±18	5	11.96±0.78**	1.29±0.31**	3.10±0.11**	0.33±0.07**
12	0	491±13	5	11.41±0.92	3.23±0.39	2.32±0.17	0.66±0.08
	0.01	477±33	5	12.02±0.78	3.10±0.41	2.52±0.11	0.65±0.10
	0.03	470±45	5	12.06±1.36	3.25±0.21	2.57±0.05	0.69±0.05
	0.1	450±22	5	13.15±1.63	1.00±0.18**	2.92±0.26**	0.22±0.04**
18	0	519±38	10	12.28±0.93	3.28±0.48	2.37±0.16	0.63±0.10
	0.01	499±30	10	12.45±1.42	3.40±1.10	2.49±0.18	0.68±0.22
	0.03	488±32	9	12.59±0.98	3.98±0.54	2.58±0.16**	0.82±0.11
	0.1	473±29*	9	14.19±1.35*	0.82±0.24**	3.00±0.13**	0.17±0.05**
Females							
6	0	230±15	5	5.41±0.14	58±5	2.36±0.14	25±2
	0.01	231±13	5	5.78±0.70	70±15	2.50±0.31	31±8
	0.03	220±23	5	5.33±0.80	61±16	2.43±0.27	27±5
	0.1	205±10	5	5.55±0.10	64±6	2.72±0.16	31±3
12	0	289±28	5	6.01±0.73	69±13	2.09±0.23	24±4
	0.01	290±27	5	6.92±1.03	64±9	2.40±0.36	22±2
	0.03	275±21	5	6.40±0.53	66±16	2.33±0.13	24±5
	0.1	223±13**	5	6.15±0.22	65±66	2.76±0.13*	29±3
18	0	365±30	8	7.60±0.88	85±12	2.08±0.15	23±2
	0.01	365±30	10	7.87±1.00	89±13	2.15±0.16	24±4
	0.03	353±44	10	7.69±0.93	78±16	2.18±0.14	22±4
	0.1	264±18**	9	7.39±0.83	75±18	2.79±0.35**	28±7

a : Number of rats tested. Values are the means±S.D. for data from all rats.
Significantly different from control : *P<0.05, **P<0.01.

- *Clinical observations: description, severity, time of onset and duration.*

No remarkable changes in general appearance were observed in any rats. Survival rates in all treated animals were comparable to those of controls. Significant suppression of body weight gain was only observed in 0.1% males from month 6 and 0.1% females from month 1. Mean food intakes per rat or per kg bw in all treated groups were comparable to those of controls. Mean efficiency of feed utilization was dosedependently decreased in both sexes.

• *sensory activity, grip strength and motor activity assessments (when available)*

No data available

• *ophthalmologic findings: incidence and severity*

No data available

• *Histopathology findings: incidence and severity:*

100 ppm (4.23 mg/kg bw/day, male)

decrease of spermatogenisis (after 12 months and hereafter), seen as testicular tubule atrophy at 12 months (in 1 out of 5, marked) and at 18 months (in 3 out of 19, severe), spermatogenic arrest at 12 months (in 1 out of 5, marked) and at 18 months (in 1 out of 19, severe) , epididymis hypospermia at 12 months (in 1 out of 5, marked) and at 18 months (in 1 out of 19, severe). (see table 7 below)

300 ppm (12.7 mg/kg bw/day, male)

decrease of spermatogenisis (after 12 months and hereafter), seen as testicular tubule atrophy at 18 months (in 1 out of 18, severe), spermatogenic arrest at 18 months (in 1 out of 18, severe), epididymis hypospermia at 18 months (in 1 out of 18, severe). (see table 7)

1000 ppm (42.3 mg/kg bw/day, male)

Decrease of spermatogenisis (after 6 months and hereafter), seen as testicular tubule atrophy at 6 months (in 5 out of 5, marked/severe), at 12 months (in 5 out of 5, severe) and at 18 months (in 19 out of 19, severe), spermatogenic arrest at 6 months (in 5 out of 5, severe), at 12 months (in 5 out of 5, severe) and at 18 months (in 19 out of 19, severe) , epididymis hypospermia at 6 months (in 5 out of 5, severe), at 12 months (in 5 out of 5, severe) and at 18 months (in 19 out of 19, severe). (see table 7). At all three time points, severe testis tubules atrophy, spermatogenic arrest and epididymis hypospermia was seen in all investigated animals

Table 7. Histological findings for male rats fed diet containing MBMBP for up to 18 months.

Findings	Group	6 months				12 months				18 months			
		0%	0.01%	0.03%	0.1%	0%	0.01%	0.03%	0.1%	0%	0.01%	0.03%	0.1%
		(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(19)	(19)	(18)	(19)
Testis													
tubules													
atrophy	±	0	0	0	0	0	0	0	0	0	1	0	0
	+	0	0	0	0	0	0	0	0	0	0	0	0
	++	0	0	0	3	1	1	0	0	2	0	0	0
	+++	0	0	0	2	0	0	0	5	0	3	1	19
spermatogenic arrest													
	++	0	0	0	0	1	1	0	0	2	0	0	0
	+++	0	0	0	5	0	0	0	5	0	1	1	19
interstitum													
interstitial cell tumor		0	0	0	0	0	0	1	0	15	11	15	0
interstitial cell hyperplasia													
	+	0	0	0	0	3	4	3	0	3	7	2	0
edema													
	+	0	0	0	1	0	0	0	0	1	0	0	1
	++	0	0	0	0	0	0	0	0	0	0	1	0
Epididymis													
hypospermia	++	0	0	0	0	1	1	0	0	2	0	0	0
	+++	0	0	0	5	0	0	0	5	0	1	1	19

( ) : No. of animals examined histologically. ± : slight, + : mild, ++ : marked, +++ : severe.

• *Haematological and clinical biochemistry findings if available:*

The authors state, that several parameters in the hematological and serum biochemical analyses, demonstrated significant alteration. However, none appeared to be of biological significance, since they did not show the same tendency throughout the experimental period and/or the degrees of change were very small (data was not shown in the report).

• *Food/water consumption*

See table 5 for food comsumption data. Suppression of body weight gain was observed in the high dose (0.1%) group, but this reduction in body weight was not associated with a reduction in food intake. Furtehermore the mean efficiency of feed utilization also decreased and were drasticially decreased in the highest dose group

• *Mortality and time to death (if occurring)*

Survivel rates in all treated animals were comparable to those of controls (See table 5). No information on when during the study the animals died.

Table 5. Mean body weight, survival rate, food intake and chemical intake for rats fed MBMBP for 18 months.

	Males				Females			
MBMBP in the diet (%)	0	0.01	0.03	0.1	0	0.01	0.03	0.1
Final mean body weight (g)	545	528	520	498	375	368	353	278
Survival rate (%)	95	95	91	95	90	100	95	95
Mean food intake (g/rat/day)	16.6	16.7	16.6	16.2	12.2	12.7	12.2	11.5
Mean food intake (g/kg BW/day)	42	42	42	42	49	51	50	54
Mean MBMBP intake (mg/kg BW/day)	0	4.2	12.7	42.3	0	5.1	15.1	54.2
Mean efficiency of feed utilization[a] (% of control)	100	93	88	68	100	95	93	43

[a] Values represent the means of body weight gain (g) per feed intake (g) at 4, 8, 12 and 16 months.

• *Necropsy findings:*

None

3.10.1.4 Study 4 Subacute study

Study reference:

Publication type: Summary article in Japanese available including English abstract and figures with English legends.

Authors: Ministry of Health and Welfare

Title of the article: Twenty-eight-day Repeat Dose Oral Toxicity Test of 2,21-Methylenebis (6-tert-butyl-p-cresol) in Rats

Year: 1996, vol. 4

Detailed study summary and results:

Test type

- *Guideline:*

Guidelines for 28-day Repeat Dose Toxicity Testing of Chemicals (Japan)

- *GLP compliant:*

Yes

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:*

It has the same CAS number as the substance in the CLH dossier

- *EC number:*

Not stated

- *CAS number:*

119-47-1

- *Degree of purity:*

>98%

- *Impurities (or a note that the impurities do not affect the classification):*

Not stated

- *Batch number:*

Not stated

Test animals

- *Species/strain/sex:*

Male and female rats; Crj:CD(SD)

- *No. of animals per sex per dose:*

n=6/sex/dose. Note: due to the inclusion of a recovery group for the control and the 800 mg/kg bw/day groups, n=12/sex/dose in the urinary measurements for the control and the 800 mg/kg bw/day group.

- *Age and weight at the study initiation:*

6 week old (both sexes), 209-235g (males), 142-164 g (females) (OECD SIDS, 2001)

Administration/exposure

• *Route of administration:*

Oral (gavage)

• *Duration and frequency of test/exposure period:*

Daily administration for 28 days.

• *Doses/concentration levels:*

0, 50, 200, 800 mg/kg bw/day test substance in 5% gum Arabic

• *Post exposure observation period*

14 days for a controle group and a 800 mg/kg group (n=6/sex/dose)

• *Control group and treatment:*

n=6/sex control rats, concurrent vehicle (5 % gum Arabic)

• *Vehicle:*

5% gum arabic solution

• *Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:*

Not stated

• *Actual dose (mg/kg bw) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable*

0, 50, 200, 800 mg/kg bw/day test substance in 5% gum arabic. Substance concentration in ppm were not stated.

• *Statistical methods*

Dunnett's or Scheffe's test for continuous data and Chi square test for quantal data (OECD SIDS, 2001)

Description of test design:

• *Parameters assessed:*

Urinary assessments (urine volume, osmotic pressure, specific gravity, pH, color, protein, glucose, ketone body, bilirubin, occult blood, urobilinogen), hematologigal assessments (Leucocyte, Erythrocyte, Hemoglobin, Hematocrit, Platelet, MCV, MCH, MCHC, Eosinophil, Neutro-Stab., Neutro-Seg., Lymphocyte, Monocyte, Prothrombin time, APTT), blood chemical assessments (

T.protein, Albumin, A/G ratio, T.bilirubin, GOT, GPT, y-GTP, ALP, T.cholesterol, Triglycerides, Phospholipids, Glucose, BUN, Creatinine, IP, Ca, Na, K, Cl) Absolute and Relative organ weight

(Brain, Heart, Lungs, Thymus, Liver, Spleen, Kidneys, Adrenals, Testes, Ovaries), histopathological assessments (Liver, Testis).

• *Estrous cycle length and pattern, sperm examination:*

No monitoration of estrous cycle. Sperm was examined by looking at testis for vacuolation (Sertoli cells), sperm retention, degeneration (step 19 spermatides), giant cell formation, nuclear vacuolation (spermatids) and germ cells (decreased)

• *Clinical observations performed and frequency:*

Not stated

Results and discussion

Describe the relevant findings.

• *Provide data on any dose-related observations:*

No mortality, clinical signs were indicated. No effects on body weights were observed in any dose group and no effects on absolute or relative testes weights.

No NOAEL was found. LOAEL 50 mg/kg bw/day

50 mg/kg bw/day

Males: Terminal body weight was not affected. A significant change was seen in one hematology endpoint out of 16 and in 1 of 19 investigated blood chemistry endpoints. See table 2 below. These are minor, but significant changes observed in total protein, which were significantly elevated by ~6% (an effect also seen in all treated groups) and haemoglobin, which were significantly decreased by 5% (an effect which was not seen at higher doses). A non-significant 14% increase in absolute and a significant 13 % increase in relative liver weights was seen, relative lung weights were 8% decreased, whereas other organ weights (brain, heart, thymus, spleen, kidney, adrenal and testes) were not affected. Histological examination of testes showed degeneration of step 19 spermatids (in 3 out of 6, mild) but no other adverse effects.

Females: Hematology and blood chemistry were not significantly affected and neither were any of the examined organ weights.

200 mg/kg bw/day

Males: Terminal body weight was not affected. Absolute and relative liver weights were significantly increased (by 25 and 19% respectively), significant changes in 2 of 16 hematology and 1 of 19 blood chemistry endpoints, seen as increase of total protein (by ~6%), Prothrombin time (by ~52%) and partial thromboplastin time (APTT) (by ~23%), non-significant changes in liver histology (in 1 out of 6, mild), sperm rention (in 6 out of 6, mild), vacuolation of Sertoli cells (in 6 out of 6, mild), degeneration of step 19 spermatids (in 6 out of 6, mild/moderate).

Females: significant increase absolute and relative liver weights (13 and 19% respectively), increased relative adrenal weights (16%) and mild changes in liver histology (in 1 out of 6). Significant changes in 1 out of 16 hematology and 1 out of 19 blood chemistry endpoints, seen as increase of partial thromboplastin time (APTT) (by ~16%) and albumin (by ~11%).

800 mg/kg bw/day

Males: increased absolute and relative liver weights (30 and 28% respectively), significant changes in 3 out of 16 hematology and 2 out of 19 blood chemistry endpoints, seen as increase of total protein (by ~6%), prothrombin time (by ~101%), partial thromboplastin time (APTT) (by ~41%), albumin (by ~9%) and platlets (by 14,8 %). Non-significant changes in liver histology (1 out of 6,mild), sperm rention (in 6 out of 6, moderate), degeneration of step 19 spermatids (in 6 out of 6, moderate), vacuolation of Sertoli cells (in 6 out of 6, mild).

Females: increase in absolute and relative liver weights (30%) and mild changes in liver histology (in 1 out of 6). Significant changes in 2 out of 16 hematology and 2 out of 19 blood chemistry endpoints, seen as an increase of prothrombin time (by ~62%) and partial thromboplastin time (APTT) (by ~33%), and a significant decrease in aspartate aminotransferase (GOT) (by ~16%) and blood urea nitrogen (BUN) (by ~19%). Albumin were non-significantly elevatet by ~8%.

800 mg/kg bw/day with recovery

Males: Increased relative liver weight (13%) but no histological effects in liver. Also a minor decrease (~4%) i absolute barin weight was observed, however no significant decrease when looking at the relative weight. Histopathology of testes showed significant effects in all investigated parameters; vacuolation of Sertoli cells (in 5 out of 6, mild), sperm retention (in 5 out of 6, moderate), degeneration of step 19 spermatids (in 5 out of 6, moderate), giant cell formation (in 4 out of 6, mild/marked), nuclear vacuolation of spermatids (in 4 out of 6, mild/moderate) and a decrease in germ cells (in 2 out of 6, mild/marked). The testicular effects were permanent, and for giant cell formation, nuclear valuolation of spermatids and decreased number of germ cells, more affected than in the 800 mg/kg bw/day group, as opposed to the effects on liver and hematology. Significant changes in 1 out of 16 hematology and 2 out of 19 blood chemistry endpoints, seen as significantly elevatet total protein (by ~6%), while mean corpuscular volume (MCV) were decreased by ~3% and γ-glutamyltransferase by ~67%. These decreases were only seen in the recovery group.

Females: Significant increase in absolute (~ 13%) and relative (~15%) liver weight, and in absolute (~8%) and relative (~9%) kidney weight. Mild changes in liver histology (in 1 out of 6). Significant changes in 1 out of 16 hematology endpoints seen as an increase of eosinophils from 0 to 1.2±1.5%. This increase were only seen in the recovery group.

Table 2 Hematological findings of rats treated orally with 2,2'-methylenebis(6-*tert*-butyl-*p*-cresol) in the twenty-eight-day repeated dose toxicity test

Item	28 days dosing groups (mg/kg)				14 days recovery groups (mg/kg)	
	0	50	200	800	0	800
Male						
No. of animals	6	6	6	6	6	6
Leucocyte ($10^2/\mu l$)	72 ± 20	67 ± 17	82 ± 16	69 ± 15	80 ± 12	83 ± 12
Erythrocyte ($10^4/\mu l$)	800 ± 18	769 ± 37	773 ± 30	772 ± 37	819 ± 37	826 ± 30
Hemoglobin (g/dl)	15.0 ± 0.3	14.3 ± 0.3*	14.5 ± 0.4	14.5 ± 0.6	14.9 ± 0.6	14.6 ± 0.5
Hematocrit (%)	47.2 ± 1.4	45.3 ± 1.1	45.3 ± 1.2	45.4 ± 1.7	47.6 ± 2.5	46 ± 1.8
Platelet ($10^4/\mu l$)	101.8 ± 7	104 ± 10.6	109.1 ± 9.1	116.9 ± 8.4*	98.8 ± 11.3	106.9 ± 18.2
MCV (fl)	59 ± 2	59 ± 2	59 ± 3	59 ± 2	58 ± 2	56 ± 2*
MCH (pg)	18.8 ± 0.5	18.6 ± 0.6	18.7 ± 0.8	18.8 ± 0.6	18.3 ± 0.5	17.7 ± 0.5
MCHC (%)	31.8 ± 0.7	31.5 ± 0.4	31.9 ± 0.3	31.9 ± 0.4	31.4 ± 0.9	31.8 ± 0.4
Eosinophil (%)	0.7 ± 1.2	0.3 ± 0.5	0.5 ± 0.5	0.2 ± 0.4	0.3 ± 0.5	0.3 ± 0.8
Neutro-Stab. (%)	0.3 ± 0.8	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.5	0.2 ± 0.4	0.0 ± 0.0
Neutro-Seg. (%)	11.8 ± 1.9	15.3 ± 4.8	13.5 ± 2.5	15.3 ± 2.7	14.0 ± 7.1	15.0 ± 6.7
Lymphocyte (%)	85.0 ± 3.3	83.0 ± 4.6	85.0 ± 2.8	82.3 ± 2.5	83.0 ± 6.5	82.5 ± 8.3
Basophil (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Monocyte (%)	2.2 ± 1.3	1.3 ± 0.8	1.0 ± 0.9	1.8 ± 1.7	2.5 ± 1.6	2.2 ± 1.3
Prothrombin time (sec)	15.9 ± 3.6	19.6 ± 3.3	24.1 ± 2.8**	31.9 ± 3.6**	14.1 ± 0.9	14.7 ± 3.1
APTT (sec)	21.6 ± 2.4	24.4 ± 3.1	26.5 ± 1.6**	30.5 ± 1.8**	21.2 ± 1.7	21.9 ± 3.0
Female						
No. of animals	6	6	6	6	6	6
Leucocyte ($10^2/\mu l$)	57 ± 16	63 ± 15	46 ± 7	61 ± 6	64 ± 18	55 ± 8
Erythrocyte ($10^4/\mu l$)	760 ± 35	745 ± 35	729 ± 63	727 ± 31	773 ± 30	746 ± 31
Hemoglobin (g/dl)	14.6 ± 0.5	14.0 ± 0.5	13.7 ± 0.4	14.1 ± 0.7	14.4 ± 0.5	14.0 ± 0.3
Hematocrit (%)	45.3 ± 2.1	43.7 ± 2.2	42.9 ± 1.3	43.6 ± 2.3	45.6 ± 2.0	44.6 ± 1.1
Platelet ($10^4/\mu l$)	108.2 ± 9.1	104.8 ± 4.6	102.3 ± 12.6	111.5 ± 8.7	100.1 ± 14.4	109.4 ± 9.8
MCV (fl)	60 ± 1	59 ± 2	59 ± 4	60 ± 1	59 ± 2	60 ± 1
MCH (pg)	19.2 ± 0.5	18.8 ± 0.7	18.9 ± 1.2	19.4 ± 0.5	18.6 ± 0.4	18.8 ± 0.4
MCHC (%)	32.2 ± 0.7	32 ± 0.7	32 ± 0.5	32.4 ± 0.6	31.6 ± 0.9	31.4 ± 0.2
Eosinophil (%)	0.2 ± 0.4	0.7 ± 0.8	0.3 ± 0.5	0.7 ± 0.8	0.0 ± 0.0	1.2 ± 1.5*
Neutro-Stab. (%)	0.3 ± 0.5	0.2 ± 0.4	0.2 ± 0.4	0.3 ± 0.8	0.0 ± 0.0	0.0 ± 0.0
Neutro-Seg. (%)	10.7 ± 3.2	16.7 ± 8.1	16.5 ± 6.5	16.8 ± 8.2	13.7 ± 4.1	14.0 ± 8.5
Lymphocyte (%)	87.8 ± 4.1	81.2 ± 7.7	81.2 ± 6.4	81.5 ± 9.1	85.3 ± 4.1	82.7 ± 8.8
Basophil (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Monocyte (%)	1.0 ± 1.3	1.3 ± 0.8	1.8 ± 1.3	0.7 ± 0.8	1.0 ± 0.9	2.2 ± 1.0
Prothrombin time (sec)	10.7 ± 0.6	10.8 ± 0.5	11.9 ± 1.1	17.3 ± 2.3**	10.6 ± 0.4	10.5 ± 0.3
APTT (sec)	18 ± 1.6	18.1 ± 1.4	20.9 ± 1.7**	24 ± 1.2**	18.6 ± 2.4	17.2 ± 0.7

*: $P<0.05$, **: $P<0.01$ (significantly different from control)
Values are mean ± S.D.

• *Body weight and body weight changes*

No significant effects on body weights were observed for any of the test group when compared with the control group . See fig. 1 for mean body weight change during the study.

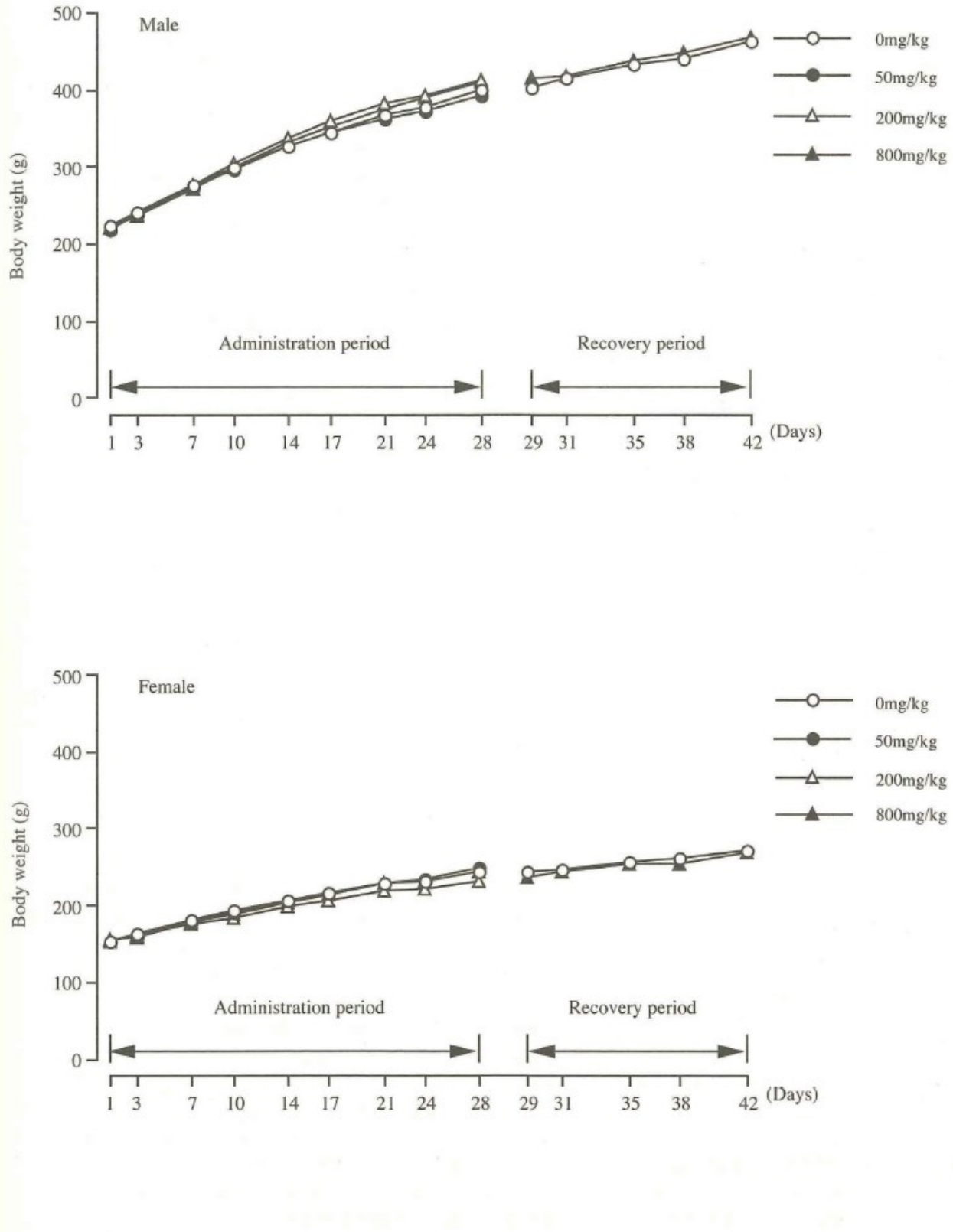


Fig. 1 Mean body weight changes of rats treated orally with 2,2'-methylenebis (6-*tert*-butyl-*p*-cresol) in the twenty-eight day repeated dose toxicity test

• *Body weight at sacrifice and absolute and relative organ weight data:*

50 mg/kg bw/day

Males: A non-significant 14% increased in absolute and a significant 13 % increase in relative liver weights was seen, relative lung weights were 8% decreased, whereas other organ weights (brain, heart, thymus, spleen, kidney adrenas and testes) were not affected.

200 mg/kg bw/day

Males: Absolute and relative liver weights were significantly increased (by 25 and 19% respectively)

Females: Significant increase absolute and relative liver weights (13 and 19% respectively), increased relative adrenal weights (16%)

800 mg/kg bw/day

Males: Increased absolute and relative liver weights (30 and 28% respectively)

Females: Increase in absolute and relative liver weights (30%)

800 mg/kg bw/day recovery

Males: Increased relative liver weight (13%). Also a minor decrease (~4%) i absolute barin weight was observed, however no significant decrease when looking at the relative weight.

Females: Significant increase in absolute (~ 13%) and relative (~15%) liver weight, and in absolute (~8%) and relative (~9%) kidney weight.

In general, no effects on body weights were observed in any dose group and no effects on absolute or relative testes weights. See table 4 below for body weight and organ weight data.

Table 4 Organ weights of rats treated orally with 2,2'-methylenebis(6-*tert*-butyl-*p*-cresol) in the twenty-eight-day repeated dose toxicity test

Item	28 days dosing groups (mg/kg)				14 days recovery groups (mg/kg)	
	0	50	200	800	0	800
Male						
No. of animals	6	6	6	6	6	6
Final Body Weight (g)	356.6 ± 21.1	359.7 ± 20.6	375.8 ± 31.4	369.4 ± 28	425.7 ± 12.7	430.4 ± 50.2
Absolute organ weight						
Brain (g)	2.04 ± 0.03	2.11 ± 0.04	2.10 ± 0.07	2.10 ± 0.12	2.17 ± 0.07	2.08 ± 0.06*
Heart (g)	1.30 ± 0.17	1.49 ± 0.13	1.35 ± 0.07	1.41 ± 0.14	1.35 ± 0.07	1.37 ± 0.09
Lungs (g)	1.33 ± 0.11	1.23 ± 0.10	1.30 ± 0.11	1.25 ± 0.09	1.31 ± 0.05	1.38 ± 0.11
Thymus (g)	0.57 ± 0.13	0.51 ± 0.06	0.56 ± 0.13	0.53 ± 0.07	0.47 ± 0.04	0.47 ± 0.16
Liver (g)	10.31 ± 0.68	11.76 ± 0.89	12.89 ± 1.45**	13.43 ± 1.78**	11.42 ± 0.63	13.16 ± 2.32
Spleen (g)	0.71 ± 0.13	0.71 ± 0.15	0.71 ± 0.09	0.68 ± 0.10	0.73 ± 0.12	0.74 ± 0.18
Kidneys (g)	2.60 ± 0.18	2.74 ± 0.21	2.74 ± 0.20	2.73 ± 0.30	2.93 ± 0.20	2.93 ± 0.41
Adrenals (mg)	68.9 ± 14.8	69.7 ± 6.4	60.3 ± 5.2	74.1 ± 11.9	69.7 ± 13.7	82.0 ± 32.3
Testes (g)	3.14 ± 0.26	3.28 ± 0.28	3.31 ± 0.23	3.33 ± 0.28	3.17 ± 0.21	2.81 ± 0.49
Relative organ weight						
Brain (g/100 gB.W.)	0.58 ± 0.04	0.59 ± 0.03	0.56 ± 0.05	0.57 ± 0.04	0.51 ± 0.02	0.49 ± 0.05
Heart (g/100 gB.W.)	0.37 ± 0.06	0.42 ± 0.02	0.36 ± 0.02	0.38 ± 0.03	0.32 ± 0.01	0.32 ± 0.03
Lungs (g/100 gB.W.)	0.37 ± 0.02	0.34 ± 0.02*	0.35 ± 0.02	0.34 ± 0.02*	0.31 ± 0.01	0.32 ± 0.02
Thymus (g/100 gB.W.)	0.16 ± 0.03	0.14 ± 0.01	0.15 ± 0.04	0.14 ± 0.02	0.11 ± 0.01	0.11 ± 0.03
Liver (g/100 gB.W.)	2.89 ± 0.15	3.27 ± 0.08**	3.43 ± 0.19**	3.62 ± 0.28**	2.68 ± 0.08	3.04 ± 0.24*
Spleen (g/100 gB.W.)	0.20 ± 0.03	0.20 ± 0.03	0.19 ± 0.03	0.18 ± 0.02	0.17 ± 0.03	0.17 ± 0.03
Kidneys (g/100 gB.W.)	0.73 ± 0.06	0.76 ± 0.02	0.73 ± 0.03	0.74 ± 0.08	0.69 ± 0.04	0.68 ± 0.04
Adrenals (mg/100 gB.W.)	19.3 ± 3.5	19.5 ± 2.5	16.1 ± 0.8	20.0 ± 2.6	16.4 ± 3.1	19.0 ± 6.4
Testes (g/100 gB.W.)	0.88 ± 0.1	0.91 ± 0.07	0.89 ± 0.08	0.91 ± 0.07	0.75 ± 0.06	0.66 ± 0.15

Female						
No. of animals	6	6	6	6	6	6
Final Body Weight (g)	225 ± 10.3	225.8 ± 13.9	212.1 ± 15.8	225.6 ± 13.7	246.5 ± 13.0	244.1 ± 15.4
Absolute organ weight						
Brain (g)	1.96 ± 0.06	1.93 ± 0.03	1.95 ± 0.03	1.97 ± 0.07	1.98 ± 0.06	1.99 ± 0.03
Heart (g)	0.90 ± 0.05	0.93 ± 0.10	0.92 ± 0.08	0.98 ± 0.14	0.88 ± 0.07	0.89 ± 0.07
Lungs (g)	1.05 ± 0.06	1.01 ± 0.08	1.02 ± 0.07	1.03 ± 0.08	1.08 ± 0.08	1.14 ± 0.14
Thymus (g)	0.52 ± 0.12	0.56 ± 0.09	0.46 ± 0.08	0.53 ± 0.11	0.50 ± 0.06	0.47 ± 0.07
Liver (g)	6.79 ± 0.35	7.35 ± 0.32	7.65 ± 0.67*	8.83 ± 0.43**	6.70 ± 0.35	7.60 ± 0.39**
Spleen (g)	0.49 ± 0.06	0.5 ± 0.05	0.43 ± 0.04	0.5 ± 0.05	0.57 ± 0.1	0.57 ± 0.05
Kidneys (g)	1.70 ± 0.12	1.82 ± 0.17	1.66 ± 0.16	1.74 ± 0.08	1.70 ± 0.08	1.84 ± 0.11*
Adrenals (mg)	73.7 ± 8.8	69.0 ± 6.1	80.7 ± 10.5	83.3 ± 9.6	73.6 ± 11.8	78.9 ± 4.8
Ovaries (mg)	92.1 ± 17.6	89.4 ± 10.7	85.5 ± 9.8	93.7 ± 12.9	92.6 ± 19.8	95.3 ± 8.3
Relative organ weight						
Brain (g/100 gB.W.)	0.87 ± 0.05	0.86 ± 0.06	0.93 ± 0.08	0.87 ± 0.04	0.80 ± 0.03	0.82 ± 0.04
Heart (g/100 gB.W.)	0.40 ± 0.03	0.41 ± 0.05	0.44 ± 0.06	0.43 ± 0.05	0.36 ± 0.03	0.37 ± 0.02
Lungs (g/100 gB.W.)	0.47 ± 0.02	0.45 ± 0.03	0.48 ± 0.04	0.46 ± 0.03	0.44 ± 0.02	0.46 ± 0.03
Thymus (g/100 gB.W.)	0.23 ± 0.05	0.25 ± 0.05	0.22 ± 0.03	0.24 ± 0.04	0.20 ± 0.03	0.19 ± 0.03
Liver (g/100 gB.W.)	3.02 ± 0.13	3.26 ± 0.16	3.61 ± 0.23**	3.92 ± 0.18**	2.72 ± 0.09	3.12 ± 0.20**
Spleen (g/100 gB.W.)	0.22 ± 0.03	0.22 ± 0.02	0.2 ± 0.01	0.22 ± 0.01	0.23 ± 0.03	0.24 ± 0.03
Kidneys (g/100 gB.W.)	0.76 ± 0.04	0.81 ± 0.05	0.78 ± 0.03	0.77 ± 0.04	0.69 ± 0.04	0.75 ± 0.03**
Adrenals (mg/100 gB.W.)	32.8 ± 3.9	30.6 ± 2.7	38.1 ± 4.0*	36.9 ± 3.4	29.9 ± 4.1	32.3 ± 1.7
Ovaries (mg/100 gB.W.)	40.8 ± 6.3	39.6 ± 3.7	40.5 ± 5.6	41.8 ± 7.1	37.4 ± 6.7	39.1 ± 3.8

*: P<0.05, **: P<0.01 (significantly different from control)
Values are mean ± S.D.

• *Clinical observations: description, severity, time of onset and duration.*

No mortality, clinical signs were indicated.

• *Sensory activity, grip strength and motor activity assessments (when available)*

No data available.

• *Ophthalmologic findings: incidence and severity*

No data available.

• *Histopathological findings: nature and severity:*

See table 5 below for histopathological findings on testis, sperm and liver effects. Note that only the liver were examined histopatologically in females.

50 mg/kg bw/day

Males: Histological examination of testes showed degeneration of step 19 spermatids (in 3 out of 6, mild) but no other adverse effects.

Female: No effects

200 mg/kg bw/day

Males: Sperm rention (in 6 out of 6, mild), vacuolation of Sertoli cells (in 6 out of 6, mild), degeneration of step 19 spermatids (in 6 out of 6, mild/moderate). Mild changes in liver histology, (1 out of 6, mild)

Females: Mild changes in liver histology, (1 out of 6, mild)

800 mg/kg bw/day

Males: Sperm rention (in 6 out of 6, moderate), degeneration of step 19 spermatids (in 6 out of 6, moderate), vacuolation of Sertoli cells (in 6 out of 6, mild). Mild changes in liver histology, (1 out of 6, mild)

Females: Mild changes in liver histology (in 2 out of 6, mild).

800 mg/kg bw/day recovery

Males: Vacuolation of Sertoli cells (in 5 out of 6, mild), sperm retention (in 5 out of 6, moderate), degeneration of step 19 spermatids (in 5 out of 6, moderate), giant cell formation (in 4 out of 6, mild/ marked), nuclear vacuolation of spermatids (in 4 out of 6, mild/moderate) and a decrease in germ cells (in 2 out of 6, mild/marked). The testicular effects were permanent, and for giant cell formation, nuclear valuolation of spermatids and decreased number of germ cells, more affected than in the 800 mg/kg bw/day group

Female: No effects

Table 5 Histopathological findings of rats treated orally with 2,2'-methylenebis(6-*tert*-butyl-*p*-cresol) in the twenty-eight-day repeated dose toxicity test

Item	28 days dosing groups (mg/kg)												14 days recovery groups (mg/kg)							
	0				50				200				800				0				800			
	–	+	++	+++	–	+	++	+++	–	+	++	+++	–	+	++	+++	–	+	++	+++	–	+	++	+++
Male																								
No. of animals	6				6				6				6				6				6			
Digestive system																								
Liver	(6)				(6)				(6)				(6)				(6)				(6)			
Hypertrophy, centrilobular	6	0	0	0	6	0	0	0	5	1	0	0	5	1	0	0	6	0	0	0	6	0	0	0
Genital system																								
Testis	(6)				(6)				(6)				(6)				(6)				(6)			
Vacuolation, Sertoli cells	6	0	0	0	6	0	0	0	0	6	0	0	0	6	0	0	6	0	0	0	1	5	0	0
Sperm retention	6	0	0	0	6	0	0	0	0	6	0	0	0	0	6	0	6	0	0	0	1	0	5	0
Degeneration, step 19 spermatids	6	0	0	0	3	3	0	0	0	5	1	0	0	0	6	0	6	0	0	0	1	1	4	0
Giant cell formation	6	0	0	0	6	0	0	0	6	0	0	0	6	0	0	0	6	0	0	0	2	2	1	1
Nuclear vacuolation, spermatids	6	0	0	0	6	0	0	0	6	0	0	0	6	0	0	0	6	0	0	0	2	3	1	0
Germ cells, decreased	6	0	0	0	6	0	0	0	6	0	0	0	6	0	0	0	6	0	0	0	4	1	0	1
Female																								
No. of animals	6				6				6				6				6				6			
Digestive system																								
Liver	(6)				(6)				(6)				(6)				(6)				(6)			
Hypertrophy, centrilobular	6	0	0	0	6	0	0	0	5	1	0	0	4	2	0	0	6	0	0	0	6	0	0	0

Grade sign: –, none; +, mild; ++, moderate; +++, marked

Figure in parentheses represents the number of animals with tissues examined histopathologically.

• *Haematological and clinical biochemistry findings if available:*

Hemoglobin

A significant 5% decrease (males) in the **50 mg/kg bw/day group**, an effect which was not seen at higher doses

Total protein (T.protein)

Total protein were significantly elevatet in males by ~6% in **all treated groups** including the recovery group.

Prothrombin time

Prothrombin time were elevated by ~52% (males) in the **200 mg/kg bw/day group** and by ~101% (males) and ~62% (females) in the **800 mg/kg bw/day group**, but not in the recovery group, where levels were not different form control group levles.

partial thromboplastin time (APTT)

Partial thromboplastin time (APTT) were significantly elevated by ~23% (males) and ~16% (females), in the 200 mg/kg bw/day group and by ~41% (males) and ~33% (females) in the **800 mg/kg bw/day group**, but not in the recovery group, where levels were not different form control group levles.

Albumin

Albumin were significantly elevatet by ~11% (females) in the **200 mg/kg bw/day group** and by ~9% (males) in the **800 mg/kg bw/day group**. Albumin were also elevated by ~8% in the female **800 mg/kg bw/day group**, but not significantly

Platlets

Platlets were significantly increases by 14,8 % (males) in the **800 mg/kg bw/day group**.

Aspartate Aminotransferase (GOT)

Aspartate Aminotransferase were significantly decreased by ~16% in the female **800 mg/kg bw/day group**

Blood urea nitrogen (BUN)

Blood urea nitrogen were significantly decreased by ~19% in the female **800 mg/kg bw/day group**.

Mean corpuscular volume (MCV)

Mean corpuscular volume were only decreased in **the recovery group** by ~3% in males

Eosinophil

Eosinophils were only increased in **the recovery group** from 0 to 1.2±1.5% in females

γ-glutamyltransferase (γ-GTP)

γ-glutamyltransferase were only decreased in **the recovery group** by ~67% in males.

See table 2 above, p.41 for haematological and table 3 below for blood chemistry findings

Table 3 Blood chemical findings of rats treated orally with 2,2'-methylenebis(6-*tert*-butyl-*p*-cresol) in the twenty-eight-day repeated dose toxicity test

Item	28 days dosing groups (mg/kg)				14 days recovery groups (mg/kg)	
	0	50	200	800	0	800
Male						
No. of animals	6	6	6	6	6	6
T.protein (g/dl)	4.8 ± 0.1	5.1 ± 0.2**	5.1 ± 0.1**	5.1 ± 0.2**	5.0 ± 0.2	5.3 ± 0.3*
Albumin (g/dl)	3.5 ± 0.1	3.6 ± 0.2	3.6 ± 0.1	3.8 ± 0.1*	3.5 ± 0.1	3.7 ± 0.2
A/G ratio	2.7 ± 0.33	2.41 ± 0.28	2.58 ± 0.27	2.95 ± 0.27	2.5 ± 0.46	2.27 ± 0.17
T.bilirubin (mg/dl)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1
GOT (IU/l)	85 ± 14	90 ± 6	82 ± 9	83 ± 7	92 ± 13	91 ± 12
GPT (IU/l)	24 ± 5	27 ± 4	24 ± 2	25 ± 5	23 ± 2	25 ± 5
γ-GTP (IU/l)	0.0 ± 0.1	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.1 ± 0.2*
ALP (IU/l)	336 ± 59	366 ± 51	328 ± 30	349 ± 50	295 ± 14	267 ± 60
T.cholesterol (mg/dl)	41 ± 7	40 ± 4	49 ± 6	45 ± 5	36 ± 9	43 ± 7
Triglycerides (mg/dl)	28 ± 10	28 ± 4	28 ± 8	24 ± 5	27 ± 6	28 ± 8
Phospholipids (mg/dl)	73 ± 10	73 ± 11	82 ± 9	78 ± 5	67 ± 12	77 ± 7
Glucose (mg/dl)	119 ± 20	118 ± 16	111 ± 6	117 ± 9	134 ± 11	135 ± 9
BUN (mg/dl)	16.3 ± 1.3	14.4 ± 1.7	14.3 ± 0.9	16.5 ± 1.6	15.7 ± 1.4	17.5 ± 2.3
Creatinine (mg/dl)	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.2
IP (mg/dl)	7.6 ± 0.5	7.6 ± 0.5	7.5 ± 0.5	7.4 ± 0.3	7.1 ± 0.3	7.1 ± 0.4
Ca (mg/dl)	9.5 ± 0.3	9.5 ± 0.2	9.5 ± 0.2	9.4 ± 0.2	9.4 ± 0.4	9.5 ± 0.3
Na (mEq/l)	145.9 ± 0.6	145.9 ± 0.9	145.2 ± 0.7	146 ± 1.0	145.7 ± 1.0	145.9 ± 0.7
K (mEq/l)	4.12 ± 0.09	4.13 ± 0.1	4.21 ± 0.08	4.21 ± 0.17	4.18 ± 0.21	4.26 ± 0.13
Cl (mEq/l)	107.3 ± 1.3	106.9 ± 0.7	107.2 ± 1.0	106.9 ± 0.9	105.7 ± 1.5	104 ± 2.2
Female						
No. of animals	6	6	6	6	6	6
T.protein (g/dl)	4.8 ± 0.3	4.9 ± 0.1	5.2 ± 0.4	5.2 ± 0.1	5.2 ± 0.4	5.2 ± 0.3
Albumin (g/dl)	3.6 ± 0.3	3.6 ± 0.2	4 ± 0.3*	3.9 ± 0.2	3.7 ± 0.3	3.8 ± 0.3
A/G ratio	2.82 ± 0.48	2.84 ± 0.51	3.29 ± 0.49	3.01 ± 0.57	2.57 ± 0.3	2.64 ± 0.27
T.bilirubin (mg/dl)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
GOT (IU/l)	96 ± 11	104 ± 10	92 ± 9	81 ± 6*	83 ± 12	77 ± 10
GPT (IU/l)	18 ± 5	19 ± 3	22 ± 3	20 ± 1	19 ± 4	18 ± 2
γ-GTP (IU/l)	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.3	0.3 ± 0.1	0.3 ± 0.2
ALP (IU/l)	223 ± 45	194 ± 27	184 ± 39	192 ± 56	188 ± 25	165 ± 25
T.cholesterol (mg/dl)	46 ± 14	42 ± 10	57 ± 6	59 ± 9	42 ± 9	49 ± 11
Triglycerides (mg/dl)	14 ± 6	12 ± 4	13 ± 4	16 ± 4	12 ± 3	13 ± 3
Phospholipids (mg/dl)	83 ± 21	75 ± 17	94 ± 11	97 ± 8	79 ± 18	88 ± 20
Glucose (mg/dl)	111 ± 16	104 ± 15	114 ± 19	99 ± 6	112 ± 22	122 ± 7
BUN (mg/dl)	20.2 ± 2.8	20 ± 3.1	20.8 ± 2.2	16.4 ± 1.8*	20.9 ± 1.7	20.7 ± 2.4
Creatinine (mg/dl)	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	0.4 ± 0.1
IP (mg/dl)	8.1 ± 0.7	8.0 ± 0.5	8.6 ± 0.7	7.8 ± 0.6	7.8 ± 1.2	8.0 ± 0.4
Ca (mg/dl)	9.5 ± 0.3	9.4 ± 0.1	9.7 ± 0.4	9.5 ± 0.3	9.7 ± 0.5	9.8 ± 0.3
Na (mEq/l)	146.8 ± 0.9	147.5 ± 1.3	147.7 ± 0.7	147.1 ± 0.9	145.9 ± 1.0	146.1 ± 1.3
K (mEq/l)	4.11 ± 0.17	4.1 ± 0.12	4.27 ± 0.25	4.05 ± 0.13	4.05 ± 0.25	4.09 ± 0.20
Cl (mEq/ll)	109.1 ± 1.9	110.2 ± 0.8	110 ± 1.1	109.7 ± 1.8	107.5 ± 1.2	108 ± 0.8

*: P<0.05, **: P<0.01 (significantly different from control)
Values are mean ± S.D.

- *Food/water consumption*

No data available

- *Mortality and time to death (if occurring)*

All animals survived, until they were sacrificed at the end of the study. Animals were terminated after 29 days and 43 days (for the recovery groups).

3.10.1.5 Study 5: Study of male reproductive toxicity with 2 months exposure time

Study reference:

Publication type: Peer reviewed publication - scientific article

Authors: Asamu Takahashi and Shinshi Oishi

Title of the article: Male reproductive toxicity of bisphenol antioxidants in mice and rats and their estrogenic effect.

journal: Archives of Toxicology

year: 2006, vol 80: 225-241

Detailed study summary and results:

Test type

- *Guideline:*

no

- *GLP compliant:*

no

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:*

It has the same CAS number as the substance in the CLH dossier. 3 other substances were investigated in the same study. DBMC is abbreviated MM in the article.

- *EC number:*

Not stated

- *CAS number:*

119-47-1

- *Degree of purity:*

>99%

- *Impurities*

Not stated

- *Batch number*

GC01 (Lot No.)

Test animals

- *Species/strain/sex*

Male mice; Crj:CD-1(ICR)

Male rats; F344/DuCrj(Fischer)

- *No. of animals per sex per dose*

n=8/dose for both mice and rats (only males)

- *Age and weight at the study initiation*

4 weeks old (mice, ~19.4g; rats, ~57.9g)

Administration/exposure

• *Route of administration:*

Oral (feed)

• *Duration and frequency of test/exposure period*

Daily administration (in diet) for 2 months

• *Doses/concentration levels, rationale for dose level selection*

Rats; 0.06% in diet. Rationale for dose level selection: According to the authors, the dose of 0.06% is a little lower than the toxic dose (0.1%) to testis when male Slc:Wistar rats were dietarily administered for 6–18 months.

Mice; 0.25 % in diet. Rationale for dose level selection:According to the authors, the dose of 0.25% is approximately equal to the minimum toxic dose of BPA to male reproductive organs in F344/DuCrj rats, but not toxic to mice

• *Post exposure observation period*

No post exposure observation period

• *Control group and treatment*

Normal diet, without the test substance.

• *Vehicle:*

None. Substance added to standard chow diet.

• *Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:*

Rats and mice were routinely fed a standard chow diet, CE-2. The experimental pellet diets were made from the powdered CE-2 and the test substance, using a mixer, a pellet maker and a dryer. The diets were kept in a refrigerator at 10˚C before use. The basal CE-2 diet was free of contamination by pesticides.

• *Actual dose (mg/kg bw) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable*

Rats; ca. 38.6-58.0 mg/kg bw/day

Mice; ca. 371-447 mg/kg bw/day

• *Statistical methods*

Variations of body and organ weights are routinely expressed as the standard deviation (SD). Bartlett's test, analysis of variance (ANOVA), Kruskal–Wallis test, and Dunnett's parametric or non-parametric test were used as tests for significance of differences. Fisher's exact test was used for histopathologic data. The limit for significance was set at $P<0.05$.

Description of test design:

• *Parameters assessed:*

Body weight and food intake were occasionally measured. Only mean values of food intake and chemical intake were described in the tables. At termination of administration, mice and rats were killed and the blood was collected. Preputial glands, testes, epididymides, prostate glands, seminal vesicles with coagulation glands, kidneys, and liver were dissected out and weighed. Spleen weight was also measured in the rat 0.6% study. Testis was fixed with formalin, sectioned, and stained routinely with hematoxylin and eosin, and observed microscopically. Serum was separated by centrifugation for the determination of testosterone.

• *Estrous cycle length and pattern, sperm examination:*

Testicular sperm content and daily sperm production (DSP) were determined by the procedure previously reported in a referred article by Takahashi and Oishi, 2003. One whole testis frozen at 80 C from each rat or mouse was reweighed and homogenized in 0.9% NaCl–Triton X-100 solution. After staining by Trypan blue, unbroken nuclei of elongated spermatids were counted on the hemocytemeter. daily sperm production and its efficiency (daily sperm production/g testis) were determined by the division of the number of spermatids per testis, and spermatids per g testis with 6.1 for rats and 4.8 for mice. Only male mice and rats in the study, thus no data on estrous cycle length and pattern.

• *Clinical observations performed and frequency:*

Clinical signs of toxicity were daily recorded if any.

Results and discussion

• *Data on dose-related observations*

40-60 mg/kg bw/day (Rat) (only one dose tested)

A non-significant, 7 %-increase in terminal body weight was observed. Liver, kidney and spleen weights were not determined. Absolut testes and epididymides weights were non-significantly decreased by 2 and 12 % respectively. Significant decrease in relative testicular (9%) and epidididymal (18 %) weights were seen and histopathological changes, including vacuolisation of sertoli cells (in 8 out of 8), disappearance of basement membrane (in 8 out of 8), degeneration of spermatids (in 7 out of 8), exfoliation (in 7 out of 8), retention (in 8 out of 8) and broken tails of elongated spermatids (in 7 out of 8). Moreover, the daily sperm production (DSP) was significant decreased in treated animals (~30 %). Serum testosterone levels were not significant changed.

2500 ppm, 371-447 mg/kg bw/day (mean of 414 mg/kg bw/day) (Mouse)

No significant changes in body weight gain, terminal body weight, absolute or relative weights of testes, sex accessory organ weights, liver and kidney. However, treated animals showed significant histopathological changes in testes, including giant cell formation (in 6 out of 8) and sloughing of seminiferous tubules (in 4 out of 8), and non-significant haemorrhage in testis (in 1 out of 8), dialated lumen of vacuolated and multinucleated spermatocytes (in 3 out of 8) and leidig cell vacuolization (in 2 out

of 8). Serum testosterone levels were not significant changed, whereas daily sperm production was not assessed.

• *Body weight and body weight changes*

No effect om mouse body weight between controle group and the 0.25 % group (See fig. 2 for mean mouse body weight change during the study). A non-significant, 7 %-increase in terminal body weight was observed for rats reciving the test substance

Note that the test substance of interest, abbreviated MM, is marked with a red box.

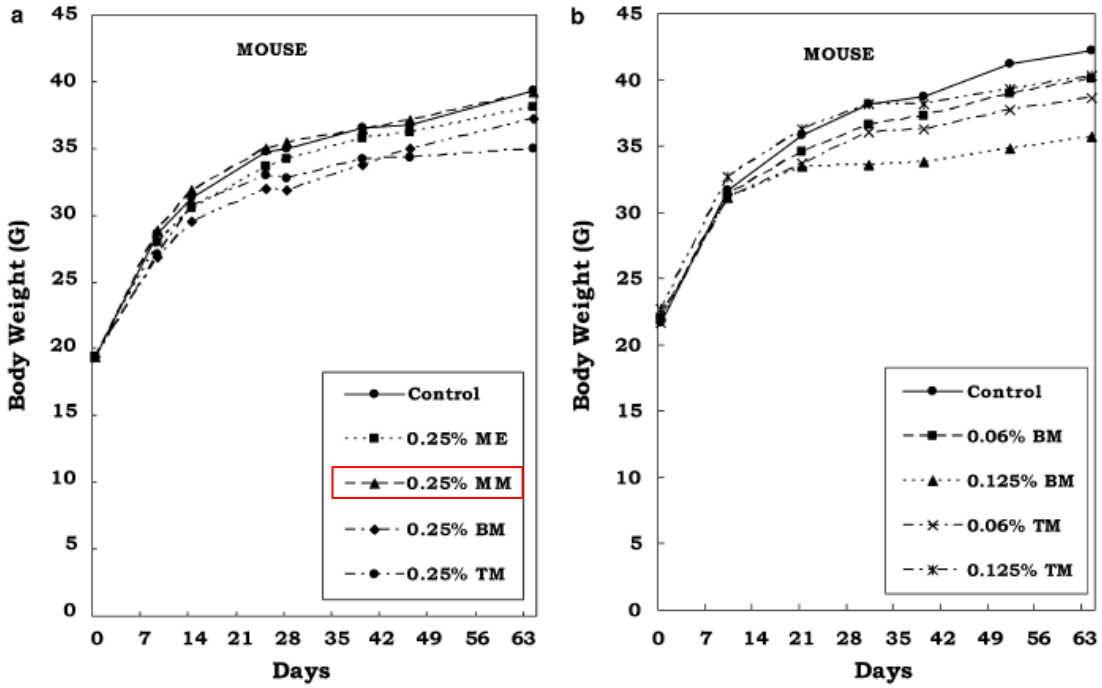


Fig. 2 Changes in mean body weight of male mice fed ME, MM, BM, or TM at a level of 0.25% (**a**), and BM or TM at levels of 0.06 and 0.125% (**b**). Body weights of 0.125% BM-treated mice after day 31 were significantly smaller than the control by Dunnett's test

• *Body weight at sacrifice and absolute and relative organ weight data:*

Mouse: No significant effect on mice terminal body weight, absolute or relative weights of liver, kidney, testes or sex accessory organ (see table 1)

Rat: A non-significant, 7 %-increase in terminal body weight was observed for rats reciving the test substance. Liver, kidney and spleen weights were not determined. Absolut testes and epididymides weights were non-significantly decreased by 2 and 12 % respectively. Significant decrease in relative testicular (9%) and epididdymal (18 %) weights were seen (see table 3)

Data on the test groups reciving the test substance of interest, are marked with a red box.

• *food/water consumption*

No significant effect on food intake for mice (see table 1) or rats (see table 3)

Table 1 Body weight, food intake, chemical intake and organ weight of male mice dietarily administered four bisphenol antioxidants for 2 months (N=8).

	Exp. 1				
	Control	0.25% ME	0.25% MM	0.25% BM	0.25% TM
Initial body weight (g)	19.4 ± 1.1	19.3 ± 1.0	19.4 ± 1.1	19.4 ± 1.0	19.4 ± 1.0
Terminal body weight (g)	39.3 ± 3.2	38.1 ± 3.6	39.2 ± 2.4	37.2 ± 4.2	35.0 ± 5.3
Mean food intake (g/kg/day)	152–186	138–179	148–183	136–180	140–198
Mean chemical intake (mg/kg/day)	–	345–447	371–457	340–450	351–495
Absolute organ weight					
Testes (mg)	258 ± 51	229 ± 27	246 ± 29	252 ± 43	252 ± 50
Epididymides (mg)	102 ± 11	95 ± 7	95 ± 7	90 ± 11*	87 ± 13**
Seminal vesicles and coagulation glands (mg)	323 ± 33	306 ± 47	319 ± 54	259 ± 49*	247 ± 70*
Prostate glands (mg)	16 ± 1	15 ± 2	14 ± 3	12 ± 2**	12 ± 3**
Preputial glands (mg)	176 ± 56	161 ± 36	155 ± 34	119 ± 54*	98 ± 33**
Liver (g)	2.20 ± 0.16	2.52 ± 0.28	2.33 ± 0.14	3.68 ± 0.57**	2.85 ± 0.38**
Kidneys (mg)	661 ± 99	627 ± 52	663 ± 85	614 ± 89	654 ± 112
Relative organ weight (per 100 g body)					
Testes (mg)	663 ± 148	607 ± 97	628 ± 55	678 ± 95	731 ± 152
Epididymides (mg)	260 ± 38	251 ± 25	242 ± 20	244 ± 30	249 ± 30
Seminal vesicles and coagulation glands (mg)	823 ± 73	802 ± 93	810 ± 105	694 ± 90*	708 ± 208
Prostate glands (mg)	41 ± 6	39 ± 6	36 ± 7	32 ± 10 *	34 ± 8
Preputial glands (mg)	445 ± 122	424 ± 93	393 ± 73	314 ± 109*	274 ± 70**
Liver (g)	5.63 ± 0.48	6.60 ± 0.23	5.94 ± 0.33	9.86 ± 0.56**	8.23 ± 1.05**
Kidneys (g)	1.69 ± 0.24	1.65 ± 0.14	1.69 ± 0.14	1.65 ± 0.13	1.87 ± 0.21

ND not determined *$P < 0.05$, **$P < 0.01$, significantly different from the appropriate control by Dunnett's

Table 3 Body weight, food intake, chemical intake and organ weight of male rats dietarily administered four bisphenol antioxidants for 2 months (N=8).

	Exp. 3				
	Control	0.06% ME	0.06% MM	0.06% BM	0.06% TM
Initial body weight (g)	57.9 ± 1.8	58.0 ± 2.5	57.9 ± 2.0	57.7 ± 2.0	57.7 ± 2.5
Terminal body weight (g)	228.6 ± 24.8	228.3 ± 29.7	245.0 ± 19.6	239.0 ± 12.0	242.4 ± 16.6
Mean food intake (g/kg/day)	68.2–99.3	68.2–92.8	64.3–96.7	67.9–96.2	68.3–95.5
Mean chemical intake (mg/kg/day)	–	40.9–55.7	38.6–58.0	40.7–57.7	41.0–57.3
Absolute organ weight					
Testes (g)	2.81 ± 0.16	2.71 ± 0.31	2.76 ± 0.09	2.88 ± 0.11	3.04 ± 2.88
Epididymides (mg)	675 ± 81	645 ± 164	593 ± 57	716 ± 55	728 ± 41
Seminal vesicles and coagulation glands (mg)	487 ± 124	452 ± 220	542 ± 101	546 ± 97	547 ± 99
Ventral prostate glands (mg)	190 ± 55	189 ± 76	230 ± 42	212 ± 37	216 ± 42
Dorsolateral prostate glands (mg)	134 ± 34	137 ± 52	161 ± 29	158 ± 32	169 ± 38
Preputial glands (mg)	167 ± 54	161 ± 46	170 ± 25	169 ± 19	177 ± 24
Liver (g)	ND	ND	ND	ND	ND
Kidneys (g)	ND	ND	ND	ND	ND
Spleen (mg)	ND	ND	ND	ND	ND
Relative organ weight (per 100 g body)					
Testes (g)	1.24 ± 0.08	1.19 ± 0.04	1.13 ± 0.09**	1.21 ± 0.04	1.21 ± 0.07
Epididymides (mg)	295 ± 16	278 ± 474	242 ± 17**	299 ± 11	300 ± 11
Seminal vesicles and coagulation glands (mg)	211 ± 35	190 ± 83	220 ± 31	227 ± 29	224 ± 34
Ventral prostate glands (mg)	81.8 ± 16.6	80.3 ± 26.9	94.0 ± 16.3	88.5 ± 13.9	88.6 ± 12.5
Dorsolateral prostate glands (mg)	57.8 ± 9.0	58.7 ± 17.5	65.2 ± 8.0	66.1 ± 11.7	68.9 ± 13.0
Preputial glands (mg)	71.9 ± 15.2	69.3 ± 13.2	69.2 ± 7.0	70.6 ± 6.7	72.9 ± 7.7
Liver (g)	ND	ND	ND	ND	ND
Kidneys (mg)	ND	ND	ND	ND	ND
Spleen (mg)	ND	ND	ND	ND	ND

ND not determined *$P < 0.05$, **$P < 0.01$, significantly different from the appropriate control by Dunnett's test

• *Clinical observations: description, severity, time of onset and duration.*

No report on clinical observations.

• *Sensory activity, grip strength and motor activity assessments (when available)*

No data available

• *Ophthalmologic findings: incidence and severity*

No data available

• *Histopathological findings: nature and severity:*

Mice: Histopathological changes in testes includes: Significant giant cell formation (in 6 out of 8) and sloughing of seminiferous tubules (in 4 out of 8).

Non-significant haemorrhage in testis (in 1 out of 8), dialated lumen of vacuolated and multinucleated spermatocytes (in 3 out of 8) and leidig cell vacuolization (in 2 out of 8). (see table 2)

Rats: Histopathological changes seminferous tubules includes: Significant vacuolisation of sertoli cells (in 8 out of 8), exfoliation (in 7 out of 8), retention (in 8 out of 8), degenerated spermatids (in 7 out of 8), disappearance of basement membrane (in 8 out of 8) and broken tails of elongated spermatids (in 7 out of 8)

Non-significant giant cell formation (in 1 out of 8), multinucleated giant cells (in 5 out of 8), Necrotic spermatocytes and/or spermatogonia (in 2 out of 8), disappearance of germ cells (in 2 out of 8) and disappearance of round spermatids (in 1 out of 8) (see table 4)

Data on the test groups reciving the test substance of interest, are marked with a red box.

Table 2 Testicular histopathology of male mice dietarily administered four bisphenol antioxidants for 2 months (

	Exp. 1				
	Control	0.25% ME	0.25% MM	0.25% BM	0.25% TM
Gross findings					
Hemorrhage in testis			1 (12.5)		
Fatty liver				8 (100)**	3 (37.5)
Disappearance of adipose tissues					5 (62.5)**
Enlargement of cecum					1 (12.5)
Seminiferous tubules					
Exfoliation				6 (75)**	5 (62.5)**
Sloughing			4 (50)*	4 (50)*	6 (75)**
Giant cells		5 (62.5)**	6 (75)**		
Granuloma				1 (12.5)	
Vacuolated and multinucleated spermatocyte				1 (12.5)	
Dilatated lumen			3 (37.5)	6 (75)**	4 (50)*
Leidig cells					
Vacuolization		2 (25)	2 (25)		4 (50)*
Proliferation	1 (12.5)	1 (12.5)		2 (25)	4 (50)*

Data are expressed as numbers of injured mice with the incidence (%) in parentheses* $P < 0.05$, ** $P < 0.01$, significantly different.

Table 4 Testicular histopathology of male rats dietarily administered four bisphenol antioxidants for 2 months ($N=8$)

	Exp. 3					Exp. 4		
	Control	0.06% ME	0.06% MM	0.06% BM	0.06% TM	Control	0.25% BM	0.25% TM
Gross findings								
Fatty liver							8 (100) **	
Enlargement of cecum								2 (25)
Seminiferous tubules								
Vacuolated Sertoli cells			8 (100) **	1 (12.5)	1 (12.5)		1 (12.5)	1 (12.5)
Exfoliation		1 (12.5)	7 (87.5)**	1 (12.5)	6 (75) **		7 (87.5)**	7 (87.5)**
Sloughing					1 (12.5)		6 (75)**	5 (62.5)**
Retention			8 (100) **					
Degenerated spermatids			7(87.5) **		2 (25)			
Giant cells			1 (12.5)					
Multinucleated giant cells	1 (12.5)		5 (62.5)					
Disappearance of basement membrane			8 (100) **					
Broken tails of elongated spermatids			7 (87.5)**		1 (12.5)			
Necrotic spermatocytes and/or spermatogonia			2 (25)					
Disappearance of germ cells			2 (25)				5 (62.5)**	2 (25)
Disappearance of round spermatids			1 (12.5)					
A degenerated tubule					1 (12.5)			1 (12.5)

Data are expressed as numbers of injured rats with the incidence (%) in parentheses** $P<0.01$, significantly different from the appropriate control by Fisher's exact test

• *Toxic or other effects on reproduction*

The daily sperm production (DSP) was measured in rats and test results showed a significant decreased in treated animals (~30 %). In mice DSP was not assessed

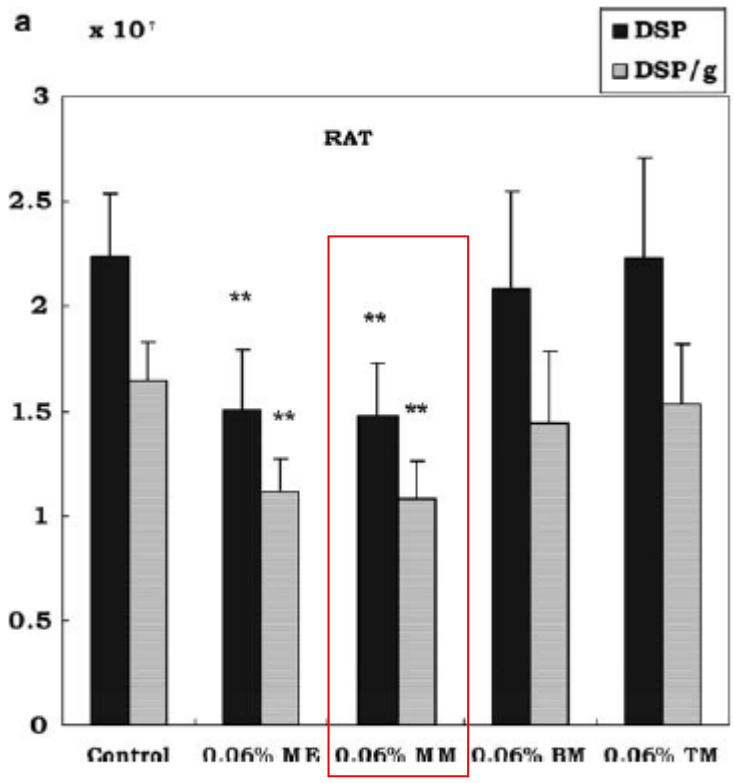


Fig. 6 DSP and DSP/g testis of male rats fed ME, MM, BM, or TM

• *Haematological and clinical biochemistry findings if available:*

Only serum testosterone levles were measured. No significant effects was observed between controle animans and animals reciving the test substance in either mice (see fig 3) or rats (see fig 7)

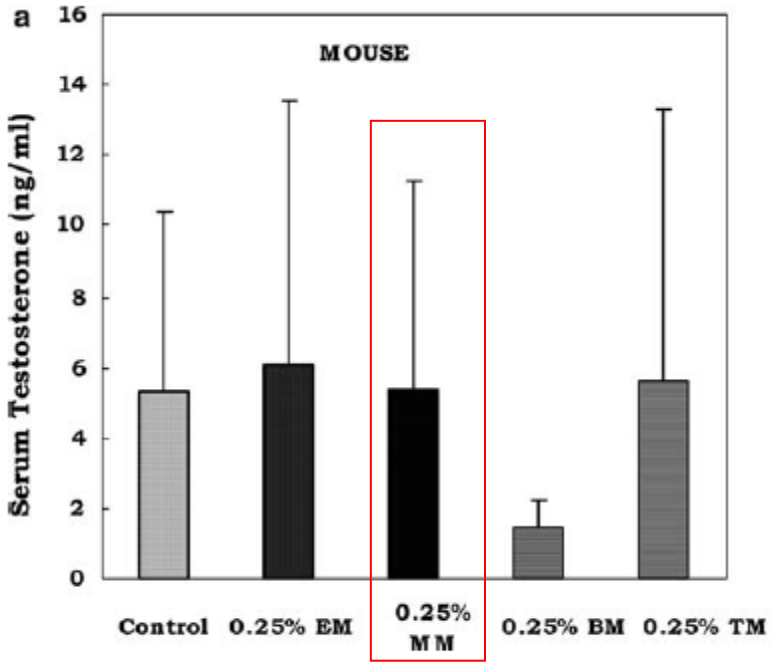


Fig. 3 Serum testosterone concentration of male mice fed ME, MM, BM and 0.125% (b)

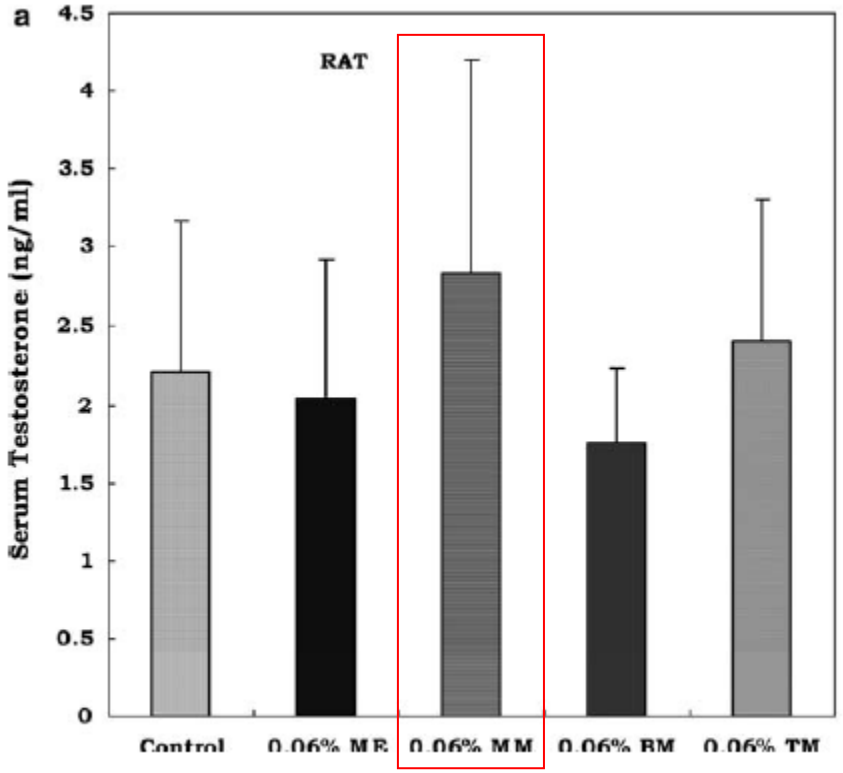


Fig. 7 Serum testosterone concentration of male rats fed ME, MM, BM, or TM

• *Mortality and time to death (if occurring)*

All animals survived, until they were sacrificed after 2 months at the end of the study.

• *Necropsy findings:*

None

3.10.1.6 Study 6: Subchronic study in the rat

Study reference:

Publication type: unpublished report - Summary article in German available including figures with English legends.

Authors: E. Bomhard, E. Löser and G. Kaliner

Title of the article: Subchroniche toxikologische untersuchungen an ratten.

year: 1982

Detailed study summary and results:

Test type

Guideline:

n.a.

- *GLP compliant:*

n.a.

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:*

Yes. The molecular formula, structural formula and molecular weight are the same as those of DBMC.

- *EC number:*

Not stated

- *CAS number:*

Not stated

- *Degree of purity:*

96-98%

- *Impurities:*

n.a.

- *Batch number:*

n.a.

Test animals

- *Species/strain/sex:*

Male and female rats; wistar

- *No. of animals per sex per dose:*

n=10/sex/dose

- *Age and weight at the study initiation:*

5-6 weeks old (both sexes) ca. 67g (males); ca 69g (females)

Administration/exposure

- *Route of administration:*

Oral (feed)

• *Duration and frequency of test/exposure period:*

Daily administration for 13 weeks

• *Doses/concentration levels, rationale for dose level selection:*

0, 100, 330, 1000 or 3000 ppm in the diet (0, 7.41, 24.91, 75.65, 281.64 mg/kg bw/day (males); 1, 9.66, 31.30, 113.16, 345.40 mg/kg bw/day (female))

• *Post exposure observation period*

Not stated

• *Control group and treatment:*

n=10/sex control rats, recived normal diet without the test substrance

• *Vehicle:*

None. Substance added to standard chow diet.

• *Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:*

Not stated

• *Actual dose (mg/kg bw) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable:*

0, 100, 330, 1000 or 3000 ppm in the diet (0, 7.41, 24.91, 75.65, 281.64 mg/kg bw/day (males); 1, 9.66, 31.30, 113.16, 345.40 mg/kg bw/day (female))

• *Statistical methods:*

Not stated

Description of test design:

• *Parameters assessed:*

Food indtake (daily meassurments), body weight (weekly meassurments), enzyme induction (cytochrome P 450 and N-Demethylase), Absolute and relative organ weight (heart, liver, kidney, adrenal and testes), Histophatology

• *Estrous cycle length and pattern, sperm examination:*

No data available

• *Clinical observations performed and frequency:*

Not stated

Results and discussion

Describe the relevant findings. If no effects occurred, explicitly note "No effects".

• *Provide data on any dose-related observations:*

No mortality or clinical signs, no relevant changes in clinical chemistry, were noted.

100 ppm (7.41 mg/kg bw/day, male; 9.66 mg/kg bw/day, female): no adverse effects

NOAEL of 330 ppm (24.91 mg/kg bw/day, male; 31,3 mg/kg bw/day, female): no adverse effects

LOAEL of 1000 ppm (75.65 mg/kg bw/day, male; 113.16 mg/kg bw/day, female)

Male: very slight, but significantly decreased body weight gain during the study, but terminal body weight was not significantly affected (2.6% increase). A non-significant increase in absolute liver weight (10%) and a significant increase in relative liver weights (7%) was seen. A very severe reduction in absolute (64%) and relative testes weights (66%) was observed along with severe atrophy of the testes

Female: decreased body weight gain during the study but no significant effect on terminal body weight. Absolute liver weights was not significantly increased (9%) whereas relative liver weight was significantly increased (13%). Histologically, no adverse effects on reproductive organs were noted.

3000 ppm (281.6 mg/kg bw/day, male; 345.4 mg/kg bw/day, female)

Male: Body weight gain was decreased during the study, but with a non-significant 7 % decrease at study termination. Small non-significant increases in absolute liver weight and significant increases in relative weights (3 and 7 % respectively) were seen. The changes in the testes were quite severe with and almost 60% decrease in relative and absolute testes weight and severe atrophy of the testes.

Female: decrease in body weight gain during the study and a small but significant reduction in body weight at sacrifice (5%). Absolute and relative liver weights were significantly increased (24 and 31 % respectively) and atrophy of both uterus horns was observed.

- *Body weight and body weight changes*

100+300ppm: no adverse effects

1000 ppm (75.65 mg/kg bw/day, male; 113.16 mg/kg bw/day, female)

Male: Very slight, but significantly decreased body weight gain during the study, but terminal body weight was not significantly affected (2.6% increase)

Female: decreased body weight gain during the study but no significant effect on terminal body weight.

3000 ppm (281.6 mg/kg bw/day, male; 345.4 mg/kg bw/day, female)

Male: Body weight gain was decreased during the study, but with a non-significant 7 % decrease at study termination.

Female: Decrease in body weight gain during the study and a small but significant reduction in body weight at sacrifice (5%)

See fig 1 for body weight data. Body weight decreases are calculated from this graph,, whereas data on statistical significance are from the CSR report.

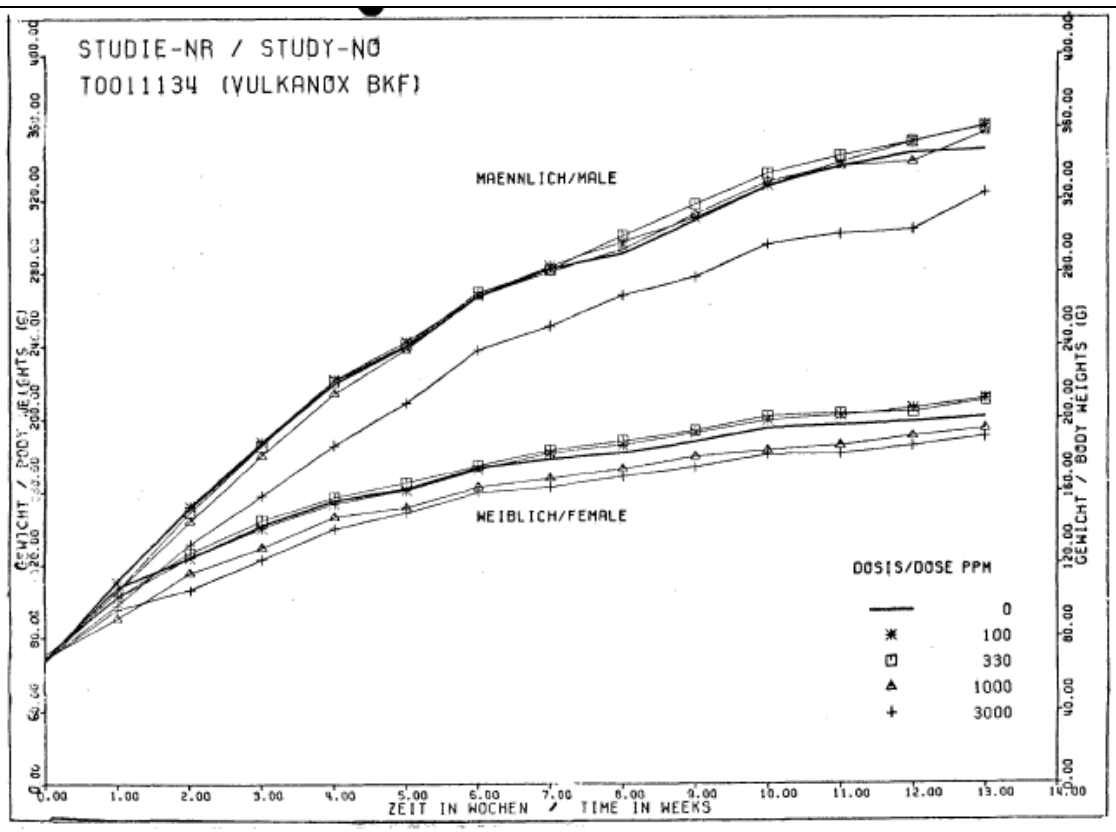


Abb. 1: Körpergewichtskurven von männlichen und weiblichen Ratten, die Vulkanox BKF 3 Monate lang mit dem Futter erhielten.

• *Body weight at sacrifice and absolute and relative organ weight data for the parental animals:*

100+300ppm: no adverse effects

1000 ppm (75.65 mg/kg bw/day, male; 113.16 mg/kg bw/day, female)

Male: Terminal body weight was not significantly affected (2.6% increase). A non-significant increase in absolute liver weight (10%) and a significant increase in relative liver weights (7%) was seen. A very severe reduction in absolute (64%) and relative testes weights (66%) was observed

Female: No significant effect on terminal body weight. Absolute liver weights was not significantly increased (9%) whereas relative liver weight was significantly increased (13%)

3000 ppm (281.6 mg/kg bw/day, male; 345.4 mg/kg bw/day, female)

Male: A non-significant 7 % decrease at study termination. Small non-significant increases in absolute liver weights and significant increases in relative weights (3 & 7 % respectively) were seen. The changes in the testes were quite severe with and almost 60% decrease in relative and absolute testes weight.

Female: A small but significant reduction in body weight at sacrifice (5%). Absolute and relative liver weights were significantly increased (24 and 31 % respectively)

See table 3 for absoloute and table 4 for relative organ weight and terminal body weight data

Tabelle 3

ABSOLUTE ORGANGEWICHTE / ABSOLUTE ORGAN WEIGHTS (MG)						
DOSE DOSIS PPM	BODY-W TIER-G (G)	HEART HERZ	LIV LEB	KIDN NIE	ADRE NN	TEST HO
MAENNLICH/MALE						
0	348	933	13595	2282	41	3335
100	361	1002	13497	2284	43	3452
330	360	1011*	14152	2333	44	3562
1000	357	1013	14950	2201	44	1183**
3000	325	935	13954	2085	39	1404**
WEIBLICH/FEMALE						
0	201	663	7445	1350	54	
100	211	677	7563	1405	54	
330	210	655	7765	1398	54	
1000	194	642	8133	1304	58	
3000	190*	658	9201**	1276	53	

Tabelle 4

RELATIVE ORGANGEWICHTE / RELATIVE ORGAN WEIGHTS (MG/100G)						
DOSE DOSIS PPM	BODY-W TIER-G (G)	HEART HERZ	LIV LEB	KIDN NIE	ADRE NN	TEST HO
MAENNLICH/MALE						
0	348	268	3900	652	12	964
100	361	277	3735	634	12	958
330	360	281	3927	648	12	990
1000	357	284*	4183*	617	12	332**
3000	325	288*	4293**	641	12	433**
WEIBLICH/FEMALE						
0	201	330	3694	672	27	
100	211	322	3599	670	26	
330	210	313	3701	666	25	
1000	194	330	4183**	671	30	
3000	190*	347	4844**	672	28	

- *Clinical observations: description, severity, time of onset and duration.*

No clinical signs were noted

- *Sensory activity, grip strength and motor activity assessments (when available)*

No data available

- *Ophthalmologic findings: incidence and severity*

No data available

- *Gross pathology findings: incidence and severity*

No gross pathology findings were noted

- *Histopathology findings: incidence and severity:*

1000 ppm (75.65 mg/kg bw/day, male; 113.16 mg/kg bw/day, female)

Male: severe atrophy of the testes Female: Histologically, no adverse effects on reproductive organs was noted **3000 ppm** (281.6 mg/kg bw/day, male; 345.4 mg/kg bw/day, female)

Male: severe atrophy of the testes (conclusion from CSR report)

Female: Atrophy of both uterus horns was observed (conclusion from CSR report)

• *Haematological and clinical biochemistry findings if available:*

No data available.

• *Food/water consumption*

Tabelle 1

	DOSIS / DOSE (PPM)				
	0	100	330	1000	3000
MAENNLICH/MALE					
FUTTERAUFNAHME / FOOD INTAKE					
G/TIER	1751	1790	1827	1800	1996
G/TIER/TAG	19	20	20	20	22
WIRKSTOFFAUFNAHME / TEST COMPOUND INTAKE					
MG/TIER		179.01	603.07	1799.70	5987.40
MG/TIER/TAG		1.97	6.63	19.78	65.80
MG/KG KGW		674.26	2266.41	6884.24	25629.31
MG/KG KGW/TAG		7.41	24.91	75.65	281.64
WEIBLICH/FEMALE					
FUTTERAUFNAHME / FOOD INTAKE					
G/TIER	1526	1506	1494	1635	1620
G/TIER/TAG	17	17	16	18	18
WIRKSTOFFAUFNAHME / TEST COMPOUND INTAKE					
MG/TIER		150.65	492.99	1635.40	4860.30
MG/TIER/TAG		1.66	5.42	17.97	53.41
MG/KG KGW		879.25	2848.75	10297.49	31431.65
MG/KG KGW/TAG		9.66	31.30	113.16	345.40

• *Mortality and time to death (if occurring)*

No mortality were noted

• *Necropsy findings:*

No necropsy findings were noted

3.10.1.7 Study 7: Subchronic study in rats

Study reference:

Publication type: unpublished repport

Authors: American Cyanamid Company - Central Medical Department

Title of the article: Report on antioxidant 2246: ninety-day repeated feeding to albino rats

year: 1965

Detailed study summary and results:

Test type

Guideline:

Not stated

- *GLP compliant:*

Not stated

Test substance

Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier

Same molecular formula, structural formula and molecular weight, but no data on CAS number or EC number.

- *EC number*

Not stated

- *CAS number*

Not stated

- *Degree of purity:*

Technical grade, 91.8% Contains 2.4%trisphenol, 1.6% mononuclear phenols (as monobutyl cresol)

- *Batch number:*

Lot No. 5704. (A 2 ¼-pound sample was received on July 9, 1965, and an additional 250-gram sample was received on June 14, 1965. Both samples were from Dr. R. L. Horton, Intermediated Department, Organic Chemicals Division (Bound Brook), and both were from Lot No.)

Test animals

- *Species/strain/sex*

Male and female rats; Carworth Farms, Nelson strain albino rats

- *No. of animals per sex per dose*

In total n=25/dose for both sexes.

Note that not all parameters were examined for alle animals:

N=5/dose/sex for hematology meassurments and promthombin time meassurments (after 3 and 13 weeks on test)

N=10/dose/sex for body weight, organ weight and food intake meassurments.

N=15/dose/sex for gross and microscopic exaninations (except for the parameter: pancreatic acinar cell shrinkage, where n=5/dose/sex)

• *Age and weight at the study initiation*

The animals were approximatly 5 veeks at the start of the study and had been in the laboratory about one veek, at the start of the feeding period. Males had a mean initial weight of 99 (84-188)g and females had a mean weight of 89 (72-109)g.

Administration/exposure

• *Route of administration:*

Oral (feed)

• *Duration and frequency of test/exposure period:*

Sub-chronic toxicity study. Daily administration for 90 days

• *Doses/concentration levels, rationale for dose level selection:*

0, 330, 1000, 3000 ppm in diet.

• *Post exposure observation period*

No post dosing observation periods stated

• *Vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water)*

None. Substance added to standard chow diet.

• *Control group and treatment*

Recived basal laboratory diet without the test substance.

• *Test substance formulation/diet preparation, achieved concentration by sex and dose level, stability and homogeneity of the preparation*

Ground purima laboratory chow (Ralston Purina Co. 1 st. Louis, Missouri) served as the basal laboratory diet. The test substance was incorporated into the diet, on a weight basis, to give the desired concentrations. Fresh diets were prepared at weekly intervals, at which time food consumption was measured for each animal.

• *Actual dose (mg/kg bw) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable*

0, 330, 1000, 3000 ppm in diet cooresponding to: 0, 25, 80.3, 241 mg/kg bw/day (males) and 0, 31.7, 92.2, 275 mg/kg bw/day (females)

• *Statistical methods*

Dunnett's method for multiple comparisons, using 2-sided comparisons. It is not clear wether this method is used on all datasets.

Description of test design:

• *Parameters assessed:*

Body weight (every second week), food indtake (weekly), promthombin time meassurments (after 3 and 13 weeks on test), haemoglobin, haematocrit, total and differential leukocyte counts, organ weight of liver, kidney, complete autopy including examination of brain and pituitary, microscopic examinations of muscle, femur with marrow, spleen, mesenteric lymph node, pancreas, liver,stomach, small intestine, large intestine, urinary bladder, gonads, prostate, uterus, seminal vesicles, adrenal, kidney, thyroid (incl. parathyroid), trachea, esophagus, lung, heart, pituitary and brain

- *Estrous cycle length and pattern, sperm examination:*

No data avalible

- *Clinical observations performed and frequency:*

All animals were under cursory observation daily and more thorough examinations were performed every second week.

Results and discussion

Describe the relevant findings. If no effects occurred, explicitly note "No effects".

- *Provide data on any dose-related observations*

NOAEL of 330 ppm (25 mg/kg bw/day, male; 31.1 mg/kg bw/day, female)

No adverse effects

LOAEL of 1000 ppm (80.3 mg/kg bw/day, male; 92.2 mg/kg bw/day, female)

Increased liver weights in males and histopathological changes in the testes (in 10 out of 14)

3000 ppm (241 mg/kg bw/day, male; 275 mg/kg bw/day, female)

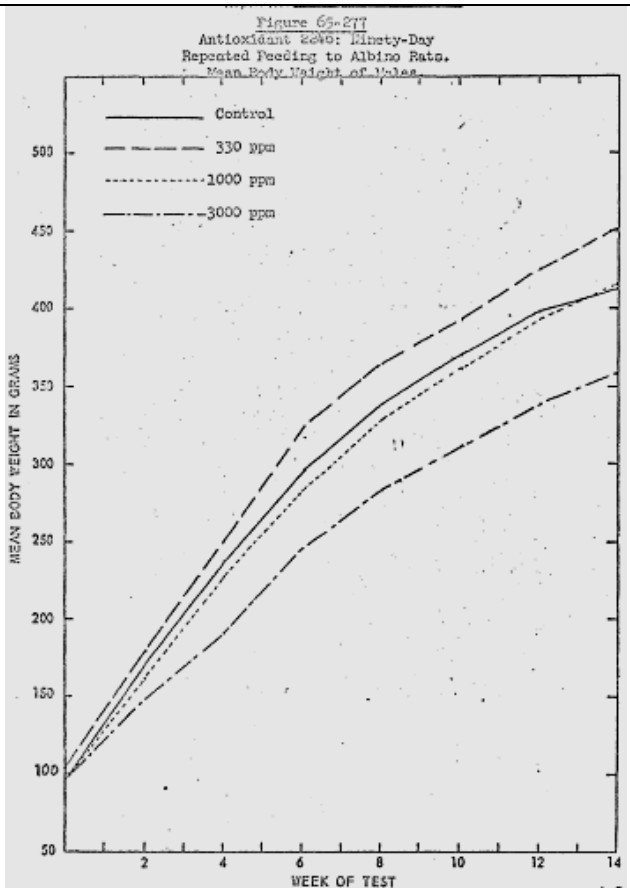
Increased liver weights in males and histopathological changes in the testes (in 14 out of 14), decreased food intake in both sexes, significant lower body weight at study termination in males, decreased kidney weight.

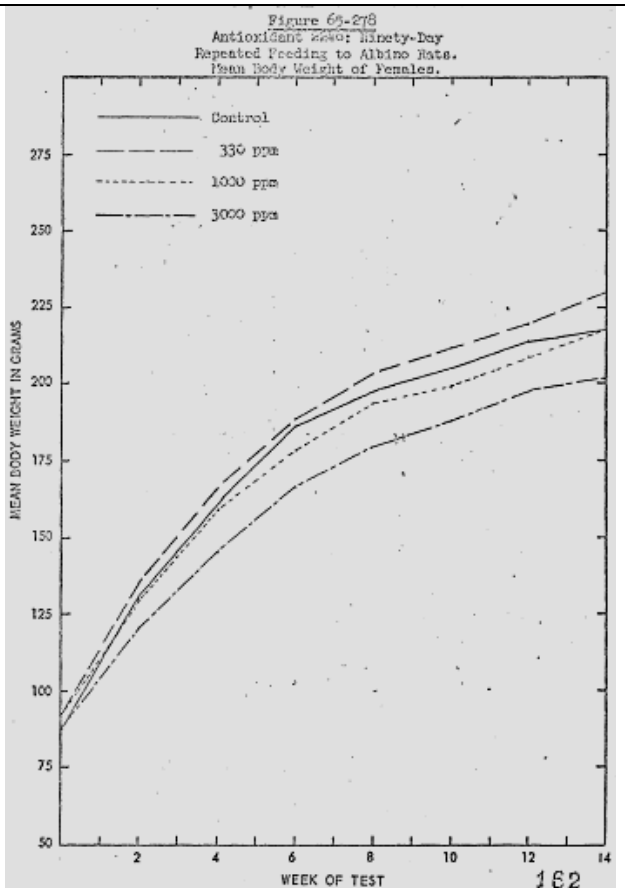
No adverse histological effects were seen in females.

- *Body weight and body weight changes*

Moderately, but significantly lower body weight at study termination in males from the 3000 ppm group. No significant difference between the other test groups and their corresponding controls.

Mean body weight curve for males are shown in fig. 65-277 and for females in fig. 65-278, both figures below.





• *Body weight at sacrifice and absolute and relative organ weight data:*

Moderately, but significantly lower body weight at study termination in males from the 3000 ppm group. No significant difference between the other test groups and their corresponding controls.

Significantly increased liver weight (in the 1000 and 3000 ppm groups) and increased kidney weight (in the 3000 ppm group) in male rats.

(se table 65-275 for males and table 65-276 for females, both figures below)

• *Food/water consumption*

Decreased food intake (g/rat/day) in the 3000 ppm group for both sexes.

(se table 65-275 for males and table 65-276 for females)

• *Haematological and clinical biochemistry findings if available:*

No values outside the acceptable ranges for percent hematocrits, hemaglobin concentrations and total leukocyte counts (stated by the authors). No statisticly significant difference between treated groups and their corresponding controls.

No significant or clinical relevant difference in prothrombin time

(se table 65-275 for males and table 65-276 for females)

Table 65-275

Antioxidant 2246:
Summary of Results of 100 Daily
Doses in the Diet of Male Albino Rats.

Concentration in diet, ppm	0	330	1000	3000
Number of animals	10	10	10	10
Mean dosage, mg/kg/day	0	25.0	80.3	241
Mean food intake, g/rat/day	20.9	21.2	20.7	18.3[a]
Mean initial weight, g/rat	97	105	98	96
Mean final weight, g/rat	416	458	419	360[a]
Mean weight gain, g/g food consumed	0.15	0.16	0.15	0.14
Mean prothrombin time, seconds[b]				
100% plasma at 3 weeks	13.48[c]	13.20[c]	13.60	13.28
100% plasma at 13 weeks	12.84	13.54	12.54	12.56
10% plasma at 13 weeks	29.20	29.72	26.76	27.32
Hematocrit, %[b]	46.10	43.10	44.10	44.50
Hemoglobin, g/100 ml[b]	15.26	14.56	14.92	15.00
Total WBC, x $10^3/mm^3$[b]	11.18	10.76	11.30	11.04
Mean liver wt, % body wt	3.11	3.39	4.05[a]	4.14[a]
Mean kidney wt, % body wt	0.65	0.66	0.64	0.72[a]

[a]Value differs significantly from control value ($p < .05$).

[b]Values based on 5 animals.

[c]Value based on 4 animals.

Table 65-276

Antioxidant 2246:
Summary of Results of 100 Daily
Doses in the Diet of Female Albino Rats.

Concentration in diet, ppm	0	330	1000	3000
Number of animals	10	10	10	10
Mean dosage, mg/kg/day	0	31.7	92.2	275
Mean food intake, g/rat/day	15.1	14.5	14.2	13.3[a]
Mean initial weight, g/rat	88	92	90	87
Mean final weight, g/rat	218	229	218	202
Mean weight gain, g/g food consumed	0.087	0.093	0.090	0.086
Mean prothrombin time, seconds[b]				
100% plasma at 3 weeks	13.28	12.84	13.26	13.24
100% plasma at 13 weeks	12.30	12.20[c]	11.42	11.20
10% plasma at 13 weeks	34.00	33.68[c]	30.90	31.16
Hematocrit, %[b]	38.90	42.30	43.90	45.50
Hemoglobin, g/100 ml[b]	13.60	14.72	14.76	15.06
Total WBC, x $10^3/mm^3$[b]	10.26	10.98	10.64	10.86
Mean liver wt, % body wt	3.64	3.28	3.82	4.08
Mean kidney wt, % body wt	0.74	0.70	0.73	0.73

[a]Value differs significantly from control value ($p \leq .05$).
[b]Values based on 5 animals.
[c]Value based on 4 animals.

• *Sensory activity, grip strength and motor activity assessments (when available).*

No data avalible

• *Ophthalmologic findings: incidence and severity*

No data avalible

• *Histopathology findings: incidence and severity:*

The authors states that two changes occurred with sufficient regularity to be of concern: Atrophy of the testes, which were seen at the two highest doses in 10 out of 14 (1000 ppm group) and in 14 out of 14 (3000 ppm group). Pancreatic acinar cell shrinkage were also seen in both males and females, however the authors state that this has also been observed in many previous occations. Since it generally occurs on the periphery of microscopic sestions and it has never shown any relationship to experimental treatment, it has been considered a processing or fixation artefact.

No substance-related lesions were encountered in female rats.

Table 65-286

Antioxidant 2246: Ninety-Day Repeated Feeding to Albino Rats.
Gross and Microscopic Lesions Occurring More than Once.

Conc. in diet, ppm	0	330	1000	3000
		MALES		
Testicular atrophy	1/15	0/15	10/14	14/14
Pancreatic acinar cell shrinkage	5/5	5/5	2/5	4/5
Suppurative bronchiectasis	-	-	-	2/14
Kidney, subcapsular mottling	-	-	-	2/14
Thyroid, dark	-	-	-	3/14
Thyroid, enlarged	-	-	-	1/4
Intestine, catarrhal inflammation	-	-	-	1/14
		FEMALES		
Ovaries red or inflammed	1/15	-	1/15	3/15
Parovarian cyst	4/15	2/15	3/15	-
Pancreatic acinar cell shrinkage	2/5	5/5	5/5	5/5
Suppurative bronchiectasis	1/15	-	-	-
Intestine, catarrhal inflammation	-	-	1/15	-
Uterus, hydrometra	-	5/15	1/15	1/15
Mes. nodes, enlargement	-	-	-	2/15
Kidney, cyst	-	-	-	2/15
Liver, tan	-	1/15	1/15	-
Thyroid, enlarged	-	-	-	1/14

• *Clinical observations: description, severity, time of onset and duration.*

A few observations were noted. Distention of the uterus with a watery mucoid fluid (hydrometra), but this were a something that the authors saw frequently in the female rats and considered to be of no concern by the authors. There were also seen some vacuoliation of individual pancreatic acini, however it was not accompanied by inflammation or other pancreatic changes. The authors states that it is generally occurring on the periphery of microscopic sections and has never shown any relationship to experimental treatment , thus it has been considered by the authors to be a processing or fixation artefact.

• *Mortality and time to death (if occurring)*

A total of 2 deaths occurred during the test period, and both of these were males: one at 3000 ppm. after 2 weeks on test, and the other at 1000 ppm after 11 weeks on test. The remaining animals were terminatet after either 3 weeks (for blood sampeling) or 13 weeks (study end)

• *Necropsy findings:*

It was not possible to determine the cause of death in either of the 2 animals which were found dead, because of autolysis. Since remaining animals were in apparent good health, it is judged that these deaths were coincitlental and unrelated to ingestion of the test substance.

3.10.1.8 Study 8: subchronic study in dogs

Study reference:

Publication type: unpublished repport

Authors: - American Cyanamid Company - Central Medical Department

Title of the article: Report on antioxidant 2246: ninety-day repeated feeding to dogs

year: 1965

Detailed study summary and results:

Test type

Guideline:

Not stated

- *GLP compliant:*

Not stated

Test substance

Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier

Same molecular formula, structural formula and molecular weight, but no data on CAS number or EC number.

- *EC number*

Not stated

- *CAS number*

Not stated

- *Degree of purity:*
- *Impurities:*

Technical grade, 91.8% real based on non-aqueous titration. Contains 2.4%trisphenol, 1.6% mononuclear phenols (as monobutyl cresol)

- *Batch number:*

Lot No. 5704. (A 2 ¼-pound sample was received on July 9, 1965, and an additional 250-gram sample was received on June 14, 1965. Both samples were from Dr. R. L. Horton, Intermediated Department, Organic Chemicals Division (Bound Brook), and both were from Lot No.)

Test animals

- *Species/strain/sex*

Male and female dogs; Purebred beagles, purchased from Green Hills Farm, Jeffersonville, New York

- *No. of animals per sex per dose*

N=2/dose for both sexes. Note that actually 2 separate feeding trials were conducted because of increased alkaline phosphatase levels in plasma and of microscopic observations

in the livers of dogs from the first trial. The first trial was conducted at dietary levels of 330 ppm,

1000 ppm and 3000 ppm, and for the second study, concentrations of 100 ppm and 200 ppm were chosen.

• *Age and weight at the study initiation*

The first study began on August 6, 1964. with dogs vhich had been born in the Febuary 28-March 25, 1964 interval. These animals were 5-6 months of age, when feeding of test diets began. The added portion of the study began on June 14, 1965. Animals used at that time were 9-12 months of age, having been born between June 9-September 9, 1964. All animals had aclimatizes at least one month prior to being placed on test, and they were in apparent good health at the start of the test periods

Administration/exposure

• *Route of administration:*

Oral (feed)

• *Duration and frequency of test/exposure period:*

Sub-chronic toxicity study. Daily administration for 90 days

• *Doses/concentration levels, rationale for dose level selection:*

A first trial was conducted at dietary levels of 330 ppm, 1000 ppm and 3000 ppm. For the second study, concentrations of 100 ppm and 200 ppm were chosen, due to increased alkaline phosphatase levels in plasma and of microscopic observations in the livers of dogs from the first trial.

• *Post exposure observation period*

• *Control group and treatment:*

Conrol group recived feed without the test-substance

• *Vehicle:*

None. Substance added to feed.

• *Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:*

Sunrise Dog Food to which 5% of vitamin supplement for fortifying dog diet and 0.1% CYPIP Diethylcarbamazine were added, served at the basal diet. Each dog were offered 250g of the appropriate diet mixture moistered with 200 ml of beef broth prepared by dissolving ~30g og MAGGI Bouillon Granules and Stock Booster in one-litter of water. One teaspoonful of MAZOLA Pure corn oil was mixed with each dog's daily ration.

No information on how the substans of interest were added to the diet, to achieved concentration, stability and homogeneity of the preparation.

• *Actual dose (mg/kg bw) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable*

A first trial was conducted at dietary levels of 330 ppm, 1000 ppm and 3000 ppm. For the second study, concentrations of 100 ppm and 200 ppm were chosen, due to increased alkaline phosphatase levels in plasma and of microscopic observations in the livers of dogs from the first trial.

• *Statistical methods*

Dunnett's method for multiple comparisons, using 2-sided comparisons at the 95 % confidence level, was used to evaluate numerical data.

Description of test design:

• *Parameters assessed:*

Body weight meassurments (every 2nd week). Hematology: Hematocrit, total hemaglobin concentration and leukocyte counts (only for the animals in trial 1, meassuered before exposure and after 14 weeks of exposure) Clinical chemistry: plasma levels of alkaline phosphatase (trial 1 animals: measured before exposure, and after 14 weeks of exposure, trial 2 animals: measured before exposure, after 12 and 17 weeks of exposure), glucose, urea nitrogen and the measurments of the ability of the liver to excrete intraveneously injected bromsulfalein (BSP) (only for the animals in trial 1, meassuered before exposure and after 14 weeks of exposure), Prothrombin time determinations (only for the animals in trial 1, meassuered before exposure (whole plasma) and after 13 and 17 weeks of exposure (whole plasma and 10 % diluted plasma in saline). Organ weights at autopsy: spleen, pancreas, liver, gonads, adrenal, kidney, thyrioid, heart, pituitary and brain. Gross and microscopic pathology of skeletal muscle, rib with marrow, spleen, mesenteric lymph node, pancreas, liver with gall bladder, stomach, ileum, caecum, colon, urinary bladder, gonads, prostate or uterus, adrenal, kidney, thyroid (with parathyroid where included), lung, heart, pituitary, and brain. All of these organs were examined from animals fed 3000 ppm of test substance. Only organs having gross lesions were examined from animals fed lower levels of the product. Since changes were encountered in the liver, this organ was examined from all animals.

• *Estrous cycle length and pattern, sperm examination:*

No data available

• *Clinical observations performed and frequency:*

General appearance was observed, no information on how frequent the dogs were examined

Results and discussion

Describe the relevant findings.

• *Provide data on any dose-related observations:*

• *Body weight and body weight changes:*

In the first trial, males and females fed 330 ppm or 1000 ppm of test substance had weight gains of 18-35%, while control animals had weight gains of 19-43%. Therefore the authors concludes that dietary level of 1000 ppm test substance or less had no adverce effect on weight gain.

In the second trial neighter control animals nor those fed 100 ppm or 200 ppm has any significant weight changes during the feeding period.

• *Body weight at sacrifice and absolute and relative organ weight data:*

No statistic data on body weight at sacrifice, but terminal body weight of treated animals do not seem to deviate much from controls. No statistical evaluation on organ weight dat due to the few number of animals per group.

• *Clinical observations: description, severity, time of onset and duration.*

A male and female (3000 ppm) died during the study. The male was noticed to be eating poorly and to be developing an abdominal swelling after 7 weeks of exposure. The feces were slightly yellow in color. A few days later, the animal had become very thin with massive ascitic distention of the abdomen. It was found dead after 58 days on test. The female began to manifest abdominal swelling (ascites) after 63 days on test. Hematology performed early suggested hemolytic or aplastic anemia. The ascites was not treated, and the animal was found dead on the 110th day of the trial.

• *Sensory activity, grip strength and motor activity assessments (when available)*

No data available

• *Ophthalmologic findings: incidence and severity*

No data available

• *Histopathology findings: incidence and severity* :

Autopsy findings form animals surviving until study termination were of varied and limited nature with the exception of the livers. Liver changes were varying from diffuse hepatitis, to marked hepatic cell disarray and misalignment without loss of cellular morphology, to bile duct epithelial hyperplasia til focal collection of hemosiderin-laden tiny macrophages arrangedin a tiny granuloma. There was neither fibrosis, nodular hyperplasia, nor neoplasia. Liver changes occurred at 330, 1000 and 3000 ppm. Furthermore in these animals hemosiderin-laden macrophages also were common in mesenteris and pancreatic lynph nodes. Ascites was not seen in any of the sacrificed animals.

• *Toxic or other effects on reproduction*

No significant effects

• *Haematological and clinical biochemistry findings if available:*

No significant difference between control and any of the treated groups in mean values of total haemoglobin concentration, of percent haematocrit, and of total leukocyte counts. No effect on glucose or urea nitrogen in blood plasma forn treated animals.

• *Food/water consumption*

The highest food refusal occurred among dogs fed the highest (3000ppm) and lowest (100ppm) dietary levels of test drug in the first trial, and among control dogs in the second trial. Overall there was no effect on food intake at dietary levels of 1000 ppm or less.

• *Mortality and time to death (if occurring)*

A male and female (3000 ppm) died during the study. The male was found dead after 58 days on trial and the female on the 110th day of the trial.

All other animals survived their respective feeding periods.

- *Necropsy findings:*

The high levle (3000 ppm) male, which was found dead on day 58, had lesions consisting of marked abdominal subcutaneous edema, pale musculature suggestive of anemia, massive ascites, a liver which was cinnamon in color and with a finely ganular surface, tiny testes, slighty swollen kidney, massive pulmonary edema, and a pale heart with left ventricular mild hypertrophy and an agonal thrombus in the right ventricle. All of these changes were modified by 18 hours of autolytic change.

Microscropically all major organs previously cited were exanined. The kidney had chronic interstitial nephritis with evidence of glomerular leakag of protein, the testes were of a juvenile type (incomplete maturity) and the liver had diffuse hepatitis. Bile ducts were unaffected, there was no hepatic necrosis, scarring or nodular hyperplasia. Liver cells were intact, mildly fatty, somewhat disarrayed. Myriads of lymphocytes and plasma cells were present in sinusoids throughout the liver.

The high level (3000 ppm) female, which was found dead on day 110 had no adominal fat, the ribs were extremely pliable, liver was slightly greyish cast and had a finely granular surface and was more nodular upon incision. The gastro-intestinal tract was emty. The uterus was minute as were the thyroids. Lungs were massively edematous with several one cm foci of early pneumonia.

Microscopically, the bone was thin and delicate, but not osteoporotic. there was marked marrow depression, the only cellular elements seen being mature erythrocyes and remnants of red cell precursors. Much of this change was autolytic. Kidneys were normal. The spleen was contracted, small, lymphoid follicles being tiny and inactive. Thyroids were aotolyzed. The liver had a thickened capsule and the liver cord pattern was broken up by dissociation and disalignment of hepatocytes. There was neither hyperplasia nor fibrosis. Lymphocytes were scattered about the paraenchyma and many kupffer cells contained a fine black pigment which stained negatively for iron.

3.10.1.9 Study 9: Developmental toxicity study in the rat

Study reference:

Publication type: Article in Japanese including English abstract and figures with English legends.

Authors: Satoru Tanaka, Kunio Kawashima, Makoto Usami, Shinsuke Nakaura, Yukio Kodama and Akira Takanaka

Title of the article: Studies on the Teratogenic Potential of 2,2'-Methylenebis (4-methyl-6-tert-butylphenol) in Rats

year: 1990

Detailed study summary and results:

Test type

Guideline:

Developmental toxicity study/teratogenicity. Study design similar to OECD Guideline 414, although not stated to be perfomed according to this guideline

- *GLP compliant:*

No (information received from the OECD SIDS, 2001).

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:*

Name stated to be 2,2'-Methylenebis (4-methyl-6-tert-butylphenol), but no further ID qualifiers.

EC number (if different from the substance identified in the CLH dossier)

Not stated.

- *CAS number (if different from the substance identified in the CLH dossier)*

Not stated.

- *Degree of purity*

Not stated.

- *Impurities (or a note that the impurities do not affect the classification)*

Not stated.

- *Batch number*

Lot No. 608008

Test animals

- *Species/strain/sex*

Female rats (Wistar)

- *No. of animals per sex per dose*

N=20-24 copulated females

- *Age and weight at the study initiation*

12-13 weeks old for female, 14 weeks old for mating males

Administration/exposure

• *Route of administration:*

Oral (gavage) (information from OECD SIDS, 2001).

• *Duration and frequency of test/exposure period*

Daily administration from the 7th to 17th day of pregnancy, with sacrifice on gastation day 20 (information from the OECD SIDS, 2001).

• *Doses/concentration levels, rationale for dose level selection*

0, 93.5, 187 or 375 mg/kg bw/day

• Control group and treatment

N=24, recived concurrent vehicle

• *Vehicle:*

Olive oil (information received from the OECD SIDS, 2001).

• *Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation*

Not stated.

• *Actual doses (mg/kg bw/day) and conversion factor from diet/drinking water test*

0, 93.5, 187 or 375 mg/kg bw/day

• *Substance concentration (ppm) to the actual dose, if applicable*

Not stated.

Description of test design:

• *Details on mating procedure (M/F ratios per cage, length of cohabitation, proof of pregnancy)*

Male/female per cage. When signs of copulation, formation of vaginal plug or sperm detection in vagina is observed in the morning, the day is determined to be Day 0 of pregnancy (information from the OECD SIDS, 2001).

• *Premating exposure period for males and females:*

None

• *Dosing schedules and pre and post dosing observation periods for P:*

Daily administration from the 7th to 17th day of pregnancy, with sacrifice on gastation day 20

• *Standardization of litters (yes/no and if yes, how and when):*

Not stated.

• *Parameters assessed for P:*

Body weight change, food consumption, mortality, No. of corpora lutea, No of implants, No. of dams with dead implants only (information OECD SIDS, 2001).

• *Estrous cycle length and pattern, sperm examination, clinical observations performed and frequency:*

No data available

• *Parameters assessed for F1:*

No. of live fetus, sex ratio, body weight, No. of dead implants (early, late), No. of fetus with external, visceral or skeletal malformation, No. of fetus with skeletal variation, fetal ossification state (information from OECD SIDS, 2001).

• *Clinical observations performed and frequency, organs examined at necropsy, others:*

General condition was observed daily (information from OECD SIDS, 2001).

• *Post exposure observation period*

None stated

Results and discussion

Describe the relevant findings. If no effects occurred, explicitly note "No effects".

• *Actual dose received by dose level by sex if known*

0, 93.5, 187 or 375 mg/kg bw/day

• *Statistical treatment of results, where appropriate*

Chi square test, t- test or rank sum test (information from OECD SIDS, 2001).

• *Provide data on any dose-related observations:*

93.5 mg/kg

No adverse effects

187 mg/kg

Diarrhea, hair fluffing, suppression of body weight gain (approximately 6% decrease in body weight on gastation day 20) and suppressed food consumption. No effects on mean number of corpora lutea, implants and live foetuses, no effects on offspring body weights and no malformations were observed.

375 mg/kg

Two dams died during the study, and diarrhoea, hair fluffing, decreased food consumption and suppression of body weight gain (appr. 16% decrease in body weight on gastation day 20) was seen. No effects on mean number of corpora lutea, no. of implants and no. of live foetuses were seen and fetal body weights were unaffected. However a non-significant increase in dead implants (69 compared to 26 in controls) and in dam with only dead implants (in 5 out of 20 compared to in 1 out of 24 in controls) was seen, and therefor offspring from only 15 litters could be examined for malformations. No foetuses showed skeletal malformations and no significant difference s were seen in no. of foetuses with variations.

For P and F1 adults (per dose):

• *Number of animals at the start of the test and matings:*

Number og dams: n=24 for the control group, n=20 for the 93.5 mg/kg bw/day group, n=20 for the 187 mg/kg bw/day group, n=22 for the 375 mg/kg bw/day group

• *Time of death during the study and whether animals survived to termination*

Two dams, which showed marked diarrhoea in the highest dose (375 mg/kg bw/day) group, died during the study (It was not stated when the animals died, during the study)

• *Body weight data for P animals selected for mating:*

93.5 mg/kg bw/day

No effect on body weight

187 mg/kg bw/day

Suppression of body weight gain (approximately 6% decrease in body weight on gastation day 20)

375 mg/kg bw/day

Suppression of body weight gain (appr. 16% decrease in body weight on gastation day 20)

(See fig. 1)

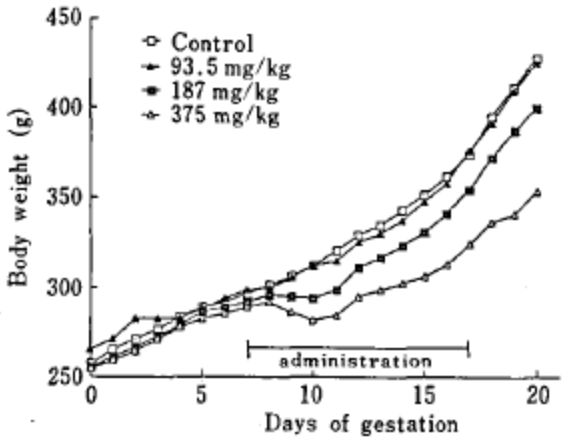


Fig. 1. Effect of oral administration of MBMBP on body weight of pregnant rats

• *Body weight at sacrifice and absolute and relative organ weight data for the parental animals:*

No data available

• *Toxic response data by sex and dose including indices of mating, fertility, gestation, birth, viabilityand lactation; indicate the numbers used in calculating the indices*

No information on indices of mating, fertility, gestation or lactation

Number of live featuses ranges from 249 to 300. No significant effect in number of live featuses (see table 2 below). However a significant increased in mean number of live featuses per litter in the 93.5 mg/kg bw/day group is found. Mean number of live featuses per litter ranges from 9.0-14.4. (see table 2 below, page 81)

• *Clinical observations: description, severity, time of onset and duration*

Two dams died during the study. Decreased food consumption, suppression of body weight gain (appr. 16% decrease in body weight on gestational day 20) and toxic signs of soiled perineal region, hair fluffing and diarrhoea, was seen for the two highest dose groups (187 mg/kg bw/day and 375 mg/kg bw/day)

• *Haematological and clinical biochemistry findings if available:*

No data available

• *Number of P and F1 females cycling normally and cycle length:*

No data available

• *Duration of gestation (calculated from day 0 of pregnancy):*

No data available

• *Precoital interval (number of days until mating and number of estrous periods until mating):*

No data available

Data on functional observations

No data available

Necropsy findings

• *Histopathological findings: nature and severity*

No data available

Body weight change and gravid uterine weight, including optionally, body weight change corrected for gravid uterine weight

No data available

Other organ weight changes if available

No data available

Number of implantations, corpora lutea, litter size:

Significant increased number of implants and increased implantation ratio in the 93.5 mg/kg bw/day group (see table 1 below). No effect on number og corpora lutea (see table 2 below, p 81). Significant increase in live foetuses in the 93.5 mg/kg bw/day group (see table 2)

Table 1. Effect of oral administration of MBMBP on pregnant rats

Dose (mg/kg)	0	93.5	187	375
No. of dams	24	20	20	22
No. of dead dams	0	0	0	2
Mortality (%)	0	0	0	9.1
No. of corpora lutea	371	313	305	296
(mean±S. D.)	(15.5±2.4)	(15.6±2.0)	(15.2±2.7)	(14.8±2.0)
No. of implants	299	300	271	249
(mean±S. D.)	(12.5±2.3)	(15.0±1.8)**	(13.5±1.7)	(12.5±2.0)
Implantation ratio (%)	82.9	96.1*	89.8	85.4

Implantation ratio is given as the average from each dam.
* (P<0.05), ** (P<0.01): Significant difference from the control value.

• *Number of pre- and post-implantation loss*

It is not stated in the abstract, tables or figures, what exactly defines early and late deaths of implants (see table 2). Thus it is not possible to tell wether early and late deaths refers to pre and post-implantation loss

• *Number of dams with abortions, early deliveries, stillbirths, resorptions and/or dead foetuses*

Only data on number of dead implants and number of dams with only dead implants. A non-significant increase was seen in dead implants (69 dead implants in the highest dose group compared to 26 dead

implants in the control group) and in dam with only dead implants (25 % in the high dose group compared to 4.2% in the controle group) (see table 2)

• *Number of live births*

Number of live featuses ranges from 249 to 300. No significant effect in number of live featuses (see table 2)

For F1 and F2 pups/litters (per dose):

• *Mean number of live pups (litter size)*

Mean number of live featuses per litter ranges from 9.0-14.4. Significant increased in mean number of live featuses per litter in the 93.5 mg/kg bw/day group. (see table 2)

• *Sex ratio*

No significant effect on sex ratio (see table 2*)*

• *Mean litter or pup weight by sex and with sexes combined.*

Mean pup body weight ranges from 3.8-3.6g (males) and 3.6-3.4g (females). A slight but significant decrease in mean male pup body weight in the 93.5 mg/kg bw/day group. No effect in other treated groups (see table 2)

Table 2. Effect of oral administration of MBMBP to pregnant rats on fetal developments

Dose (mg/kg)	0	93.5	187	373
No. of dams	24	20	20	20
No. of implants	299	300	271	249
No. of live fetuses	273	288	264	180
(mean±S. D.)	(11.4±3.4)	(14.4±2.0)*	(13.2±1.7)	(9.0±5.7)
Sex ratio (%)	102.2	88.2	88.6	95.7
(male/female)	(138/135)	(135/153)	(124/140)	(88/92)
Body weight (g)				
Male(mean±S. D.)	3.8±0.3	3.6±0.3*	3.7±0.3	3.7±0.4
Female(mean±S. D.)	3.6±0.3	3.4±0.3	3.4±0.3	3.5±0.4
No. of dead implants	26	12	7	69
Early death	20	12	7	68
Late death	6	0	0	1
Mortality (%)	8.6	4.1	2.4	28.4
No. of dams with dead implants only (%)	1(4.2)	0	0	5(25.0)
No. of fetuses with external malformation	0	0	0	0

Fetal mortality (%)is given as the average of incidence in each litter.
* (P<0.05): Significant difference from the control value.

• *Viability index (pups surviving 4 days/total births)*

No data available

Survival index at weaning

No data available

External, soft tissue and skeletal malformations and other relevant alterations

No significant differences in dilation of renal pelvis of the foetuses (See table 3). No foetuses with skeletal malformations (See table 4 below),

Table 3. Effect of oral administration of MBMBP to pregnant rat on internal organ development of the fetuses

Dose (mg/kg)	0	93.5	187	375
No. of dams	23	20	20	15
No. of fetuses examined	102	115	94	66
Dilatation of renal pelvis	0	1(1.3)	0	2(3.1)

Occurence rate (%) in parenthesis is given as the average of incidence in each litter.

Table 4. Effect of oral administration of MBMBP to pregnant rat on skeletal development of the fetuses

Dose (mg/kg)	0	93.5	187	375
No. of dams	23	20	20	15
No. of fetuses examined	171	173	170	114
No. of fetuses with skeletal malformation	0	0	0	0
No. of fetuses with variations				
Cervical ribs	1(0.7)	0	3(1.6)	2(1.7)
Varied cervical centra	1(0.7)	0	0	0
Varied thoracic centra	1(0.7)	1(0.5)	1(0.5)	1(0.8)
Shortined 13th ribs	1(0.7)	0	0	0
Varied sternebrae	62(35.9)	92(53.5)	91(52.9)	75(66.7)
Lumbar ribs	84(48.4)	90(53.6)	91(53.4)	89(77.4)
rudimentary	82(47.5)	81(48.2)	82(48.2)	86(74.7)
extra (14th rib)	2(0.9)	9(5.4)	9(5.2)	3(2.7)
27-pre-sacral vertabrae	0	0	1(0.6)	0
Ossification state (average number)				
Metacarpi	7.9	7.8	7.9	7.7
Metatarsi	8.0	8.0	8.0	8.0
Sacro-caudal vertebrae	7.8	7.8	7.8	7.7

Occurence rate (%) in parenthesis is given as the average of incidence in each litter.
** ($P<0.01$): Significant difference from the control value.

• *Number and percent of fetuses and litters with malformations (including runts) and/or variations as well as description and incidences of malformations and main variations (and/or retardations):*

No significant differences were seen in number of foetuses with skeletal variations and no significalt differences in ossification state

• *Data on physical landmarks in pups and other postnatal developmental data*

No data available

Data on functional observations

No data available

3.11 Specific target organ toxicity – single exposure

Not assessed.

3.12 Specific target organ toxicity – repeated exposure

Studies on repeated dose toxicity are describer under the end-point of reproductive toxicity above.

3.13 Aspiration hazard

4 ENVIRONMENTAL HAZARDS

Not assessed.