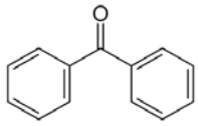
Cal/OSHA Draft IH Substance Summary for the March 5, 2019 HEAC Meeting

Substance name: Benzophenone

CAS: 119-61-9 **MW: 182.22**

Synonyms: Diphenylmethanone, Phenyl ketone, Benzoylbenzene, Diphenyl ketone, α-Oxodiphenyl methane

Molecular formula: $C_{13}H_{10}O$ Structural formula:



Conversion factors at 25 °C and 760 mm/Hg: 1 ppm = 7.45 mg/m^3

Physical appearance at room temp: white, flaked/crystalline solid

Boiling Point: 305 °C

Melting Point: 48.5 °C

Vapor Pressure: 0.006 mmHg at 48 °C

Solubility: insoluble in water, soluble in organics such as alcohol, ether, chloroform

Special physical characteristics if any: rose or geranium-like odor

Flammability and other hazards: Flash point 144°C (closed cup)

Uses/applications: It is a naturally occurring compound used in flavoring and perfumes. It is used as fixative for heavy perfumes in soaps, detergents, and room deodorizers. It is used as a flavoring agent, ultraviolet absorber in inks and coatings, and as a polymerization inhibitor for styrene. It is used in the manufacture of antihistamines, hypnotics, and insecticides.

OELs

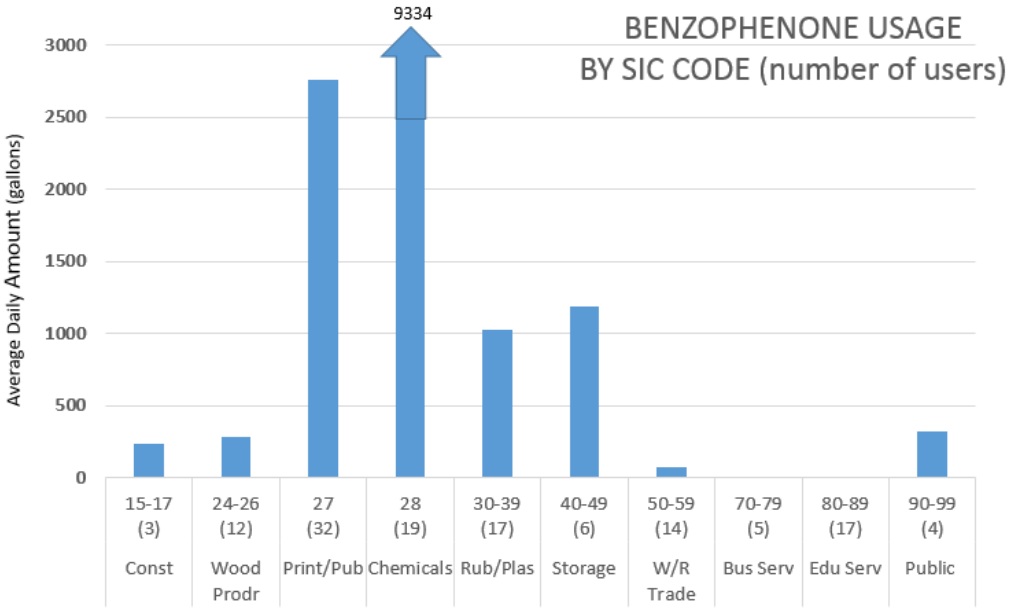
OARS-WEEL**:** 0.5 mg/m^3

Other Recommendations

Source	Findings/Recommendations	Basis/source/ref(s)	Discussion and Assessment
Prop 65 (2012)	Listed		
NTP (2006)	Some evidence of carcinogenic activity in male F344/N rats and male & female B6C3F mice; equivocal evidence of carcinogenic activity in female F344/N rats	NTP TR 533 NIH Publication No. 06-4469	
IARC (2013)	Possibly carcinogenic to humans (Group 2B)	IARC Monographs 101-007	

Usage information: EPA Inventory Update Reporting (IUR), other sources

The CalEPA CERS database reports approximately 129 users in California.



Measurement information

OSHA Method: OSHA PV2130

The detection limit of the overall procedure is 0.27 µg and the reliable quantitation limit is 0.89 µg. The equivalent air concentrations are 5.63 µg/m^3 and 18.5 µg/m^3 respectively based on the recommended sampling parameter of 240 min at 0.2L/min (48 L).

Based on this information, there are no anticipated concerns with analytical feasibility.

Recommended Workplace Controls

Providing suitable control measures such as ventilation to control exposure can be accomplished using existing equipment as most systems have the ability to control to the proposed levels.

Reproductive and developmental toxicity:

The National Toxicology Program (NTP) conducted several range-finding developmental toxicity studies with benzophenone in rats and rabbits in which effects on the foetus were only observed in the presence of maternal toxicity (NTP 2002; NTP 2004). https://ntp.niehs.nih.gov/testing/types/dev/abstracts/ter99001/ter99001.html

NTP, 2002. Developmental Toxicity Evaluation for Benzophenone (CAS No. 119-61- 9) Administered by Gavage to Sprague-Dawley (CD) Rats on Gestational Days 6 through 19. NTP Study No. TER-98-005.

Benzophenone was administered by gavage to rats on gestational days (GD) 6 to 19 at doses of 0, 100, 200, or 300 mg/kg bw/ day. Maternal toxicity was observed at all doses, including clinical signs (lethargy, piloerection, weight loss) and significantly increased maternal liver and kidney weights. Decreased fetal body weight was noted at the highest dose, and what were considered "mild developmental delays with a high probability of recovery during early postnatal development" were observed at all doses. The incidences of unossified sternebrae were increased in all dose groups and the incidence of extra rib was increased in the two highest dose groups. The maternal toxicity lowest-observed-adverse-effect level (LOAEL) was reported as 100 mg/kg bw/day (no NOAEL was established).

NTP, 2004. Developmental Toxicity Evaluation for Benzophenone (CAS No. 119-61-9) Administered by Gavage to New Zealand White Rabbits on Gestational Days 6 through 29. Final Study Report. NTP Study No. TER-99-001.

Developmental toxicity of benzophenone was investigated in rabbits, administered benzophenone by gavage in doses of 0, 5, 25 and 45 mg/kg bw per day on gestational days 6–29. Maternal body weights and feed consumption decreased in a dose-related manner, but no effects on liver and kidney weights were observed. There were no effects on prenatal viability. However, the number of successful deliveries was decreased with increasing dose of benzophenone. Fetal body weight was significantly decreased in the highest dose group. In rabbits, dose-related increases in the incidences of abortion or early delivery were reported in the mid- and high-dose groups, along with dose-related reductions in maternal body weight (not reversed) and fetal body weight. The LOAEL was considered to be 25 mg/kg bw/day for maternal toxicity and early termination of pregnancy and NOAEL was determined to be 5 mg/kg bw/day. The conclusion by the authors was that developmental toxicity was only noted in the presence of maternal toxicity.

https://ntp.niehs.nih.gov/testing/types/dev/abstracts/ter99001/ter99001.html

Hoshino N, Tani E, Wako Y, Takahashi K. 2005. A two-generation reproductive toxicity study of benzophenone in rats. J Toxicol Sci. 30:5-20.

https://www.jstage.jst.go.jp/article/jts/30/Special/30_Special_S5/_pdf/-char/en

No reproductive toxicity or effects on endocrine system were apparent in a two-generation study in which both sexes of SD rats were exposed to benzophenone in diet at 0, 6/9, 29/40 and 130/170 mg/kg bw/day (male/female). However, in both sexes of F0 and F1 parents, inhibition of body weight gain and food consumption, significantly elevated renal weights and changes in renal tubules, and significantly increased hepatic weight and centrilobular hepatocytic hypertrophy were observed in mid- and high-dose groups. In both generations, no effects were observed on male and female reproduction (sperm analysis, oestrous cycle, serum levels of testosterone, oestradiol, follicle-stimulating hormone (FSH) and luteinising hormone (LH), mating and fertility index, gestational length, number of implantation sites, number of offspring at birth and sex ratio). No effect of treatment was found on viability, physical development, including vaginal opening and preputial separation of the penis, results of reflex and response tests or on external abnormalities. Anogenital distance (AGD) was statistically significantly decreased in the low- and mid-dose group in females of the F1 generation, but not in

the high dose or in the F2 generation or in males. The decrease in F1 female AGD was up to 11%, statistically significant and based on reasonable numbers (n = 22–24) that accounted for the litter effect. A decreased female AGD may be adverse and could be an indication of developmental and/or endocrine consequences. However, the effect on AGD was not dose-dependent and no effects on fertility were observed. Data from this study presented in the following tables.

Reproductive and Developmental Effects of BP in rats

Benzophenone (ppm)	0 (Control)	100	450	2000
F0 parents / F1 offspring				
No. of pairs	24	24	24	24
No. of days until copulation (days) [b]	2.0 ± 1.2 [a]	2.2 ± 1.3	2.3 ± 1.1	2.0 ± 0.9
Mating index (%) [c]	95.8	100.0	95.8	100.0
Fertility index (%) [d]	100.0	95.8	100.0	100.0
Gestation length (days) [e]	22.0 ± 0.5	22.1 ± 0.4	22.3 ± 0.4	22.2 ± 0.4
Gestation index (%) [f]	100.0	95.7	100.0	100.0
Birth index (%) [g]	93.88	96.17	92.85	93.38
No. of implantation sites	15.0 ± 1.6	15.2 ± 1.3	15.3 ± 1.3	13.9 ± 2.3
Total No. of offspring at birth	14.0 ± 1.9	14.6 ± 1.4	14.5 ± 1.9	13.2 ± 2.3
No. of offspring born alive	14.0 ± 1.9	14.6 ± 1.4	14.2 ± 1.9	12.9 ± 2.4
Sex ratio [h]	0.52	0.55	0.51	0.46
Viability index (%)				
Day 0 [i]	100.00 ± 0.00	100.00 ± 0.00	98.03 ± 5.00	98.01 ± 6.65
Day 4 [j]	99.14 ± 2.29	98.68 ± 2.91	99.45 ± 1.82	98.73 ± 3.01
Day 21 [k]	99.46 ± 2.61	99.43 ± 2.67	100.00 ± 0.00	100.00 ± 0.00
F1 parents / F2 offspring				
No. of pairs	22	22	23	24
No. of days until copulation (days)	2.4 ± 0.9	2.6 ± 1.2	2.5 ± 1.7	2.6 ± 1.3
Mating index (%)	86.4	100.0	100.0	95.8
Fertility index (%)	84.2	95.5	100.0	100.0
Gestation length (days)	22.0 ± 0.4	22.2 ± 0.4	22.2 ± 0.4	22.4 ± 0.5
Gestation index (%)	100.0	100.0	100.0	100.0
Birth index (%)	95.42	88.98	91.39	90.79
No. of implantation sites	14.6 ± 1.8	16.0 ± 2.1	15.3 ± 2.1	14.5 ± 1.6
Total No. of offspring at birth	14.1 ± 1.8	14.6 ± 3.5	14.3 ± 1.8	13.8 ± 1.6
No. of offspring born alive	13.9 ± 1.8	14.2 ± 3.5	13.9 ± 1.8	13.1 ± 1.5
Sex ratio	0.48	0.53	0.50	0.54
Viability index (%)				
Day 0	98.74 ± 3.74	97.12 ± 4.54	96.99 ± 3.55	95.22 ± 7.99
Day 4	99.58 ± 1.68	98.45 ± 2.86	98.91 ± 3.02	97.74 ± 4.97
Day 21	100.00 ± 0.00	99.40 ± 2.73	100.00 ± 0.00	100.00 ± 0.00

Table 2. Estrous cycle.

Benzophenone (ppm)	0(Control)	100	450	2000
F0 females				
No. of females examined	24	24	24	24
Mean estrous cycle (days)	4.08 ± 0.23 [a]	4.14 ± 0.34	4.09 ± 0.25	4.04 ± 0.21
No. of females with an abnormal estrous cycle [b]	0/24	1/24	1/24	1/24
F1 females				
No. of females examined	23	22	23	24
Length of estrous cycles (days)	4.00 ± 0.00	4.15 ± 0.33	4.12 ± 0.30	4.00 ± 0.00
No. of females with an abnormal estrous cycle	1/23	2/22	0/23	2/24

Table 4: Data on sperm and hormone parameters

Benzophenone (ppm)	0(Control)	100	450	2000
F0 parents				
Sperm examination				
No. of males examined	10	10	10	10
Sperm motility (%)	92.4 ± 4.74 a	93.1 ± 5.30	95.1 ± 3.45	92.1 ± 3.96
Homogenization-resistant spermatids (× 10^6/g) (spermatid head counts in the testis)	112.9 ± 20.69	–	–	105.5 ± 16.24
Sperm Count (× 10^6/g), (cauda epididymal)	487.5 ± 175.89	–	–	530.3 ± 171.79
Abnormal sperm (%)	0.6 ± 0.46	–	–	0.3 ± 0.42
Tailless sperm (%)	2.1 ± 3.03	–	–	1.7 ± 2.00
Hormone levels				
No. of males examined	6	6	6	6
Testosterone (ng/mL)	1.47 ± 0.52	2.94 ± 1.76	2.95 ± 1.79	3.65 ± 1.17
FSH (ng/mL)	8.84 ± 1.45	9.99 ± 1.99	8.94 ± 1.28	9.89 ± 0.91
LH (ng/mL)	1.69 ± 0.13	1.73 ± 0.28	1.54 ± 0.29	1.59 ± 0.24
No. of females examined	6	6	6	6
FSH (ng/mL)	6.51 ± 1.19	6.38 ± 0.92	6.08 ± 1.52	6.94 ± 0.56
LH (ng/mL)	1.86 ± 0.13	1.79 ± 0.39	1.80 ± 0.15	1.83 ± 0.30
Estradiol (pg/mL)	69.0 ± 23.0	72.8 ± 13.7	82.8 ± 28.0	96.5 ± 34.6
F1 parents				
Sperm examination				
No. of males examined	10	10	10	10
Sperm motility (%)	94.5 ± 4.90	93.6 ± 4.14	94.9 ± 3.63	92.2 ± 3.58
Homogenization-resistant spermatids (× 10^6/g) (spermatid head counts in the testis)	139.3 ± 46.18	–	–	108.4 ± 26.42
Sperm Count (× 10^6/g), (cauda epididymal)	584.0 ± 175.53	–	–	664.8 ± 109.93
Abnormal sperm (%)	0.9 ± 1.02	–	–	1.0 ± 0.83
Tailless sperm (%)	1.9 ± 1.85	–	–	0.6 ± 0.70
Hormone levels				
No. of males examined	6	6	6	6
Testosterone (ng/mL)	3.51 ± 1.26	3.59 ± 1.52	2.98 ± 1.18	2.70 ± 1.36
FSH (ng/mL)	9.16 ± 2.42	9.20 ± 1.11	7.65 ± 0.53	9.19 ± 1.46
LH (ng/mL)	1.78 ± 0.49	1.80 ± 0.50	1.49 ± 0.25	1.60 ± 0.44
No. of females examined	6	6	6	6
FSH (ng/mL)	5.88 ± 1.03	6.44 ± 0.39	6.32 ± 0.66	5.86 ± 0.82
LH (ng/mL)	1.53 ± 0.38	1.87 ± 0.22	1.98 ± 0.49	1.72 ± 0.17
Estradiol (pg/mL)	85.0 ± 26.9	85.7 ± 25.5	93.5 ± 16.7	95.2 ± 49.7

Table 5: Data for anogenital distance, reflex/response and external examination of off-spring

Benzophenone (ppm)	0(Control)	100	450	2000
F1 pups				
No. of males examined	23	22	23	24
AGD [b] (mm) [c]	4.267 ± 0.400 [a]	4.182 ± 0.413	4.193 ± 0.433	4.150 ± 0.376
AGD/BW $^{1/3}$ [d]	1.911 ± 0.157	1.870 ± 0.172	1.873 ± 0.178	1.865 ± 0.157
No. of females examined	23	22	23	24
AGD (mm)	2.177 ± 0.179	2.014 ± 0.188*	1.959 ± 0.208**	2.055 ± 0.286
AGD/BW $^{1/3}$	0.993 ± 0.086	0.919 ± 0.080*	0.886 ± 0.082**	0.936 ± 0.125
Reflex / response (males)				
Pain response (%)	100	100	100	100
Midair righting reflex (%)	100	100	100	100
Negative geotaxis (%)	100	100	100	100
Pinna reflex (%)	100	100	100	100
Reflex / response (females)				
Pain response (%)	100	100	100	100
Midair righting reflex (%)	100	100	100	100
Negative geotaxis (%)	100	98.9	100	100
Pinna reflex (%)	100	100	100	100
External examination (males)				
No anomaly	169 / 23 [e]	175 / 22	171 / 23	145 / 24
External examination (females)				
No anomaly	154 / 23	146 / 22	162 / 23	171 / 23
Omphalocele	0 / 0	0 / 0	0 / 0	1 / 1
F2 pups				
No. of males examined	16	21	23	23
AGD (mm)	4.149 ± 0.416	4.089 ± 0.415	4.077 ± 0.265	3.968 ± 0.227
AGD/BW $^{1/3}$	1.855 ± 0.119	1.821 ± 0.142	1.817 ± 0.106	1.767 ± 0.067
No. of females examined	16	21	23	23
AGD (mm)	1.918 ± 0.152	1.955 ± 0.199	1.967 ± 0.169	1.882 ± 0.133
AGD/BW $^{1/3}$	0.870 ± 0.052	0.879 ± 0.077	0.890 ± 0.082	0.857 ± 0.057
Reflex / response (males)				
Pain response (%)	100	100	100	100
Midair righting reflex (%)	100	100	100	100
Negative geotaxis (%)	100	100	100	100
Pinna reflex (%)	100	100	100	100
Reflex / response (females)				
Pain response (%)	100	100	100	100
Midair righting reflex (%)	100	100	100	100
Negative geotaxis (%)	100	100	100	100
Pinna reflex (%)	100	100	100	100
External examination (males)				
No anomaly	109 / 16	161 / 21	166 / 23	172 / 23
External examination (females)				
No anomaly	115 / 15	145 / 21	164 / 23	145 / 23
Rudimentary tail	1 / 1	0 / 0	0 / 0	0 / 0

Additional two-generation reproductive toxicity studies were performed in rats which utilizing extra parameters to detect endocrine-disrupting activity, AGD included. Rats were given benzophenone via the diet at concentrations of 0, 100, 450 and 2,000 ppm feed (equal to 9, 40.5, and 180 mg/kg bw per day). According to the authors, no obvious effects on endocrine system and reproductive toxicological effects were detected in the F0 and F1 parents of F1 and F2 offspring (data not shown, Yamasaki et al., 2005; https://www.jstage.jst.go.jp/article/jts/30/Special/30_Special_S1/_pdf/-char/en.)

Carcinogenicity

NTP TECHNICAL REPORT ON THE TOXICOLOGY AND CARCINOGENESIS STUDIES OF BENZOPHENONE (CAS NO. 119-61-9) IN F344/N RATS AND B6C3F1 MICE (FEED STUDIES). 2006. https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr533.pdf

Two-year study in Rats: Groups of 50 male and 50 female rats were fed diets containing 0, 312, 625, or 1,250 ppm benzophenone (equivalent to average daily doses of approximately 15, 30, and 60 mg benzophenone/kg body weight to males and 15, 30, and 65 mg/kg to females) for 105 weeks. There was a positive trend in the incidences of renal tubule adenoma in males, and the incidences in 625 and 1,250 ppm males exceeded the historical control range for all routes; these neoplasms were accompanied by significantly increased incidences of renal tubule hyperplasia. The incidences of pelvic transitional epithelium hyperplasia and the severity of nephropathy were significantly increased in all exposed groups of male rats. Increased incidences of mononuclear cell leukemia in all exposed groups of females exceeded the historical control range from feed studies, and the incidence in 625 ppm females was significantly greater than that in the controls. Male rats exposed to 312 or 625 ppm had significantly increased incidences of mononuclear cell leukemia. One 625 ppm female and two 1,250 ppm females had histiocytic sarcomas, and the incidence in the 1,250 ppm group exceeded the range in the historical controls. Incidences of mammary gland fibroadenoma in females exposed to 625 or 1,250 ppm were lower than expected after adjusting for body weight.

Two-year study in Mice: Groups of 50 male and 50 female mice were fed diets containing 0, 312, 625, or 1,250 ppm benzophenone (equivalent to average daily doses of approximately 40, 80, and 160 mg/kg body weight to males and 35, 70, and 150 mg/kg to females) for 105 weeks. In male mice, there were significantly increased incidences of hepatocellular adenoma in the 625 and 1,250 ppm groups, and these incidences exceeded the historical control range. All hepatocellular neoplasms combined occurred with a positive trend. In female mice, the incidences of hepatocellular adenoma in the 625 and 1,250 ppm groups were higher than expected after adjusting for the lower body weights in these groups. Incidences of centrilobular hepatocyte hypertrophy were significantly increased in all exposed groups of males and females. The incidence of histiocytic sarcoma in 625 ppm females was significantly increased and exceeded the historical control range. The incidences of kidney nephropathy and mineralization in exposed groups of females and the severity of nephropathy in exposed groups of males were significantly increased. Study details and significant findings are outlined in Tables 1 and 2.

1.

Rat - male				
Renal Tubule, Hyperplasia	3 (1.0)	11* (1.3)	30** (1.8)	40** (2.1)•
Nephropathy	50 (1.3)	45 (2.4)•	50 (3.3)•	50 (3.8)•
Renal Tubule, Adenoma	2/50 P=0.004	2/50 P=0.688	7/50 P=0.093	8/50 P=0.017
Mononuclear Cell Leukemia	27/50 P=0.508	41/50 P=0.003	39/50 P=0.005	24/50 P=0.454
Rat - female				
Female - Renal Tubule, Hyperplasia	1 (1.0)	8* (1.5)	10** (2.2)	7* (2.0)
Female -nephrop	47 (1.1)	49 (1.4)	48 (1/7)	49 (2.0)
MOUSE - male				
Hepatocyte, Centrilobular, Hypertrophy	0	44** (2.0)	50** (2.0)	48** (3.0)
Hepatocellular Adenoma, Carcinoma, or Hepatoblastoma	18/50 P=0.013	20/50 P=0.434	25/50 P=0.118	29/50 P=0.027
Mononuclear Cell Leukemia	19/50 P=0.058	25/50 P=0.25	30/50 P=0.048	29/50 P=0.068
MOUSE - female				
Hepatocyte, Centrilobular, Hypertrophy	0	29** (2.0)	44** (2.0)	37** (2.9)
Hepatocellular Adenoma or Carcinoma	5/50 P=0.081	5/50 P=0.624N	10/50 P=0.131	9/50 P=0.165
Histiocytic Sarcoma[g]	0/50 P=0.074	0/50 can't	1/50 P=0.516	2/50 P=0.251

*** Significantly different (P≤0.05) from the control group by the Poly-3 test ** P≤0.01. Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidences are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.**

2.

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1 Mice	Female B6C3F1 Mice
Concentrations in feed	0, 312, 625, 1,250 ppm	0, 312, 625, 1,250 ppm	0, 312, 625, 1,250 ppm	0, 312, 625, 1,250 ppm
Body weights	625 and 1,250 ppm groups less than the control group	625 and 1,250 ppm groups less than the control group	Exposed groups similar to the control group	312, 625, and 1,250 ppm groups less than the control group
Nonneoplastic effects	Kidney: renal tubule, hyperplasia (standard evaluation - 1/50, 5/50, 20/50, 23/50; standard and extended evaluations combined - 3/50, 11/50, 30/50, 40/50); pelvis, transitional epithelium, hyperplasia (1/50, 11/50, 29/50, 34/50); severity of nephropathy (1.3, 2.4, 3.3, 3.8) Liver: hepatocyte, centrilobular, hypertrophy (0/50, 17/50, 31/50, 19/50); degeneration, cystic (8/50, 11/50, 20/50, 15/50)	Kidney: renal tubule, hyperplasia (standard evaluation - 0/50, 1/50, 1/50, 1/50; standard and extended evaluations combined - 1/50, 8/50, 10/50, 7/50); severity of nephropathy - (1.1, 1.4, 1.7, 2.0) Liver: hepatocyte, centrilobular, hypertrophy (0/50, 27/50, 30/50, 33/50); bile duct, hyperplasia (10/50, 35/50, 39/50, 40/50	Liver: hepatocyte, centrilobular, hypertrophy (0/50, 44/50, 50/50, 48/50); hepatocyte, multinucleated (0/50, 41/50, 47/50, 48/50); inflammation, chronic active (33/50, 47/50, 44/50, 42/50); hepatocyte, degeneration, cystic (0/50, 0/50, 5/50, 30/50) Kidney: severity of nephropathy (1.2, 1.4, 1.7, 3.0) Nose: olfactory epithelium, metaplasia (0/50, 2/50, 2/50, 24/50) Spleen: lymphoid follicle, hyperplasia, lymphoid (17/50, 31/50, 34/50, 32/50)	Liver: hepatocyte, centrilobular, hypertrophy (0/50, 29/50, 44/50, 37/50) Kidney: nephropathy (21/50, 33/50, 31/50, 30/50); mineralization (15/50, 31/50, 36/50, 49/50); severity of nephropathy - (1.2, 1.1, 1.5, 1.7) Nose: olfactory epithelium, metaplasia (0/50, 0/50, 0/50, 39/50) Spleen: lymphoid follicle, hyperplasia, lymphoid (24/50, 36/50, 37/50, 22/50)
Neoplastic effects	Kidney: renal tubule, adenoma (standard evaluation - 1/50, 1/50, 2/50, 4/50; standard and extended evaluations combined - 2/50, 2/50, 7/50, 8/50)	None	Liver: hepatocellular adenoma (11/50, 15/50, 23/50, 23/50); hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (18/50, 20/50, 25/50, 29/50)	Histiocytic sarcoma: (0/50, 0/50, 5/50, 3/50)
Equivocal findings	Mononuclear cell leukemia: (27/50, 41/50, 39/50, 24/50)	Mononuclear cell leukemia: (19/50, 25/50, 30/50, 29/50) Histiocytic sarcoma: (0/50, 0/50, 1/50, 2/50)	None	Liver: hepatocellular adenoma (5/50, 4/50, 10/50, 8/50)

The conclusions by NTP on the carcinogenicity of benzophenone were: some evidence in male rats based on the incidence of renal tubule adenoma; equivocal evidence in female rats based on the marginal increased incidence of mononuclear cell leukaemia (MNCL) and histiocytic sarcoma; some evidence in male mice based on the increased incidence of hepatocellular adenoma; some evidence in female mice based on increased incidence of histiocytic sarcoma.). A marginal increase in the incidence of histiocytic sarcoma in high dose female rats was also reported (exceeded historical control). However, both the mononuclear cell leukemia and histiocytic sarcoma observed in female rats were considered by NTP (2006) as equivocal evidence of carcinogenic activity of benzophenone

Benzophenone induced cancer on multiple sites in both rats and mice in oral chronic studies. However, the mode of action of carcinogenicity of benzophenone in the oral studies is uncertain. Given the results of the NTP bioassay and the evidence of effects on the endocrine system, IARC (2013) suspected that multiple mechanisms, such as the generation of reactive oxygen species and interference with endocrine system via multiple receptors, might be involved in the carcinogenicity of benzophenone. In addition, the pathogenesis of benzophenone-induced renal tubule cancer has not been determined by NTP (2006). While IARC (2013) considered that the short survival of high-dose male rats was attributable to the increased severity of chronic progressive nephropathy (CPN), it did not conclude that CPN was a mechanism for renal tumour development. IARC concluded that benzophenone is possibly carcinogenic to humans (2B).

Mutagenicity:

From NTP, 2006:

Benzophenone was not mutagenic in the standard Ames test using various strains of Salmonella typhimurium (Mortelmans et al., 1986) or in the Escherichia coli Pol A assay (Fluck et al., 1976). In addition, negative results were reported with benzophenone in the mouse lymphoma L5178Y/tk+/- cell test for induction of trifluorothymidine resistance (CCRIS, 1991). All three of these in vitro assays were performed with and without rodent liver S9 metabolic activation enzymes. Results of a recent investigation of the genotoxic potential of benzophenone showed no induction of DNA damage as measured by umu gene expression in S. typhimurium strain TA1535/pSK1002 in the absence or the presence of microsomes from rat, mouse, or human, however significant dose-related increases in umu gene expression were elicited in the presence of recombinant human cytochrome P450s in the same studies (Takemoto et al., 2002). In vivo, benzophenone did not increase the frequency of micronuclei in erythrocytes from bone marrow or from peripheral blood in mice after intraperitoneal injections at 200 to 500 mg/kg bw/day for 3 days or after dietary exposure at 200 to 4200 mg/kg bw/day for 14 weeks (NTP 2006). Benzophenone has been classified as non-genotoxic by several authoritative bodies.

NTP has evaluated the umu gene expression findings: “The positive results reported for benzophenone in the umu gene expression assay do not directly conflict with the negative results obtained in Salmonella gene mutation assays because the endpoints measured by the two assays differ, as do important aspects of the test protocols. Briefly, the umu assay indirectly detects DNA damage induced anywhere in the Salmonella genome by analyzing fluorescent signals produced by expression of the umu-beta-galactosidase gene complex carried in the pSK1002 plasmid (genes in the umu operon control SOS error-prone DNA repair which is expressed in response to induced damage). The Salmonella assay, in contrast, measures fixed damage induced specifically within defined regions of the histidine operon, resulting in heritable changes in the bacterial DNA directly observable as mutant colonies.

Primary DNA damage, such as that detected in the umu assay, may or may not result in mutation. In addition to the endpoint differences, the activation systems contained different liver enzyme mixtures, and the human cytochrome preparations used in the umu assay had specific enzymatic cofactors added to the mixture to ensure the availability of a sufficient number of electrons for metabolic activities to proceed. The pretreatments used to induce rodent S9 liver enzymes in standard bacterial mutation assays may not induce the P450 2A6 and specific other cytochromes that were shown to be effective in transforming benzophenone into a DNA damaging agent in the umu assay."

Table A.1: Genotoxicity data on benzophenone [FL-no: 07.032] evaluated by JECFA (2002) and considered by EFSA in FGE.69 (2008)

Chemical name FL-no JECFA-no	End-point	Test system	Concentration	Results	Reference	Comments
Benzophenone 07.032 831	Bacterial reverse mutation	*S.* Typhimurium TA97, TA98, TA100, TA1535 and TA1537	3–1,000 μg/plate	Negative[(a)]	Mortelmans et al. (1986)	Reliable with the following restriction: the study complied with current recommendations with the exception that tester strains TA102 or *E. coli* WP2*uvrA* were not used
(a): With and without metabolic activation.						
Benzophenone 07.032 831	SOS/umuC assay	*S.* Typhimurium TA1535	0–1,000 μM[(a)]	Positive	Takemoto et al. (2002)	Study is reliable. Positive at the higher concentrations (100–1,000 μM) in the presence of metabolic activation. However, the relevance of this endpoint is low
			7.8–1,000 μg/mL[(a)]	Positive	Kotnik et al. (2016)	Study is reliable. Positive at the highest concentration in the presence of metabolic activation. However, the relevance of this endpoint is low
	Bacterial reverse mutation assay	*S.* Typhimurium TA98, TA100, TA1535, TA1537	10–2,000 μg/plate[(a),(d)]	Negative	CCRIS (2009)	Reliability cannot be evaluated (full study report not available)
			3–333 μg/plate[(b),(e)]	Negative		
			10–1,000 μg/plate[(b),(e)]	Negative		
			1–166 μg/plate[(c),(e)]	Negative		
	Gene mutation in mammalian cells	L5178Y (tk+/−) mouse lymphoma cells	33–90 μg/mL[(c)] 35–145 μg/mL[(b)]	Negative		
			8.9–142.8 μg/mL[(c)] 8.9–141.7 μg/mL[(b)]	Negative	Jeon et al. (2007)	Reliable with limitations (experimental details are not provided) 80% inhibitory concentration (IC_{80}) was used as maximum concentration

Table B.2: *In vivo* genotoxicity studies on benzophenone [FL-no: 07.032]

Chemical name FL-no JECFA-no	Test system *in vivo*	Test object	Route	Dose	Result	Reference	Comments
Benzophenone 07.032 831	Micronucleus assay in bone marrow	B6C3F1 male mice	Intraperitoneal	200, 300, 400, 500 mg/kg bw (solvent: corn oil)	Negative	NTP (2006)	Reliable without restriction. Three injections at 24 h intervals; sacrifice 24 h after 3rd injection. No toxicity to the bone marrow
	Micronucleus assay in peripheral blood polychromatic erythrocytes	B6C3F1 male and female mice	Oral (feed)	1,250, 2,500, 5,000, 10,000, 20,000 ppm	Negative		Reliable without restriction. Harvest at end of 14-week dosing regimen. No toxicity to the bone marrow
		Male CBA mice	Intraperitoneal	500, 1,000, 2,000 mg/kg bw	Negative	Abramsson-Zetterberg and Svensson (2011)	Reliable without restriction. Single intraperitoneal injection, peripheral blood sampled after 42 h
		Male NMRI mice		100, 250, 400, 600 mg/kg bw	Negative		Reliable without restriction. Single intraperitoneal injection, peripheral blood sampled after 42 h

Endocrine effects:

Benzophenone had no affinity for the human ER receptor, did not activate ER-mediated gene transcription, did not activate progesterone-mediated gene transcription and had no proliferative effect on MCF—7 cells (human breast cell line). In animal studies, no estrogenic effects of benzophenone have been observed (see Table). Several long-term rodent studies, including a 2-generation reproductive toxicity study, which did not detect effects in some endpoints sensitive to endocrine disruption (i.e. anogenital distance F1 and F2 offspring, timing of sexual maturation in F1 offspring, weights and histopathological evaluation of testes, epididymis, prostates, seminal vesicles, ovaries and uterus in F1 parental animals, and levels of testosterone, FSH, LH and estradiol, estrous cyclicity and semen quality in F0 and F1 animals). The metabolite of benzophenone, 4-BP, has demonstrated estrogenic effects in in vitro assays and animal studies. A recent study (Kerdivel, 2013) confirmed that BP was not estrogenic in MCF-7 cells were as 4-OH-BP was.

Results of studies on mammalian endocrine and reproductive systems

Animal species	Administration method	Administration period	Dose	Results	References
Rat (SD, female) 6 rats/group	s.c. (Uterotrophic assay) (Ovariectomized rats, Ovariectomized at the age of 6 weeks)	BZP was administered for 7 days from the age of 8 weeks, and uterus was removed on the 8th day and weighed.	BZP 0, 5, 50, 500 mg/kg/day	Slight increase in uterine weight at 500 mg/kg/day. (estrogenic effect)	CERI, 2001b
			BZP 0, 5, 50, 500 mg/kg/day + 17 α -Ethinylestradiol 0.3 μg/kg/day (s.c.)	Slight decrease in uterine weight at 50 mg/kg/day or above. (anti-estrogenic effect)	
Rat (SD, female) 6 rats/group	s.c. (Uterotrophic assay)	BZP was administered for 3 days from postnatal day 20, and the uterus was removed on the 4th day and weighed.	BZP 0, 2, 20, 200 mg/kg/day	No effect on uterine weight.	CERI, 2001a
Rat (SD, female)	s.c. (Uterotrophic assay)	BZP derivatives were administered for 3 days from postnatal day 21, and the uterus was removed 6 hr after the final dose and weighed.	4-Hydroxy-BZP at 0, 100, 200 and 400 mg/kg/day	The uterine weight increased dose-dependently. (estrogenic effect)	Nakagawa & Tayama, 2001
			Benzhydrol 400 mg/kg/day	No effect on uterine weight.	
Rat (SD, male) 6 rats/group	Oral gavage (Hershberger assay) (Castrated rats, castrated at the age of 6 weeks)	BZP was administered for 10 days from the age of 7 weeks, and male accessory reproductive organs were weighed on the 11th day.	BZP 0, 1, 10, 100 mg/kg/day	No effect on weights of male accessory reproductive organs.	CERI, 2001b
			BZP 0, 1, 10, 100 mg/kg/day + Testosterone propionate 0.4 mg/kg/day (s.c.)	No effect on weights of male accessory reproductive organs.	

Endocrine Assay for BP (BZP in table), 4-OH-BP (metabolite) and BP derivatives

Item	Test methods and conditions	Results	Conclusion	References
ER binding assay	Human ER binding assay (recombinant ERα ligand domain)	BZP: IC50 value: $>10^{-4}$M (E2: 1.7×10^{-9} M)	BZP has no binding affinity for human ER.	CERI, 2001a
		BZP derivatives (IC50 value) 4-Hydroxy-BZP: 1.3×10^{-5} M (E2: 1.4×10^{-9} M; RBA: 0.011%) 3-Hydroxy-BZP: 1.0×10^{-5} M (E2: 1.3×10^{-9} M; RBA: 0.013%) 4,4'-Dihydroxy-BZP: 7.3×10^{-6} M (E2: 1.2×10^{-9} M; RBA: 0.017%) 2,4-Dihydroxy-BZP: 8.9×10^{-6} M (E2: 1.2×10^{-9} M; RBA: 0.014%) 2,4,4'-Trihydroxy-BZP: 1.7×10^{-6} M (E2: 1.2×10^{-9} M; RBA: 0.074%) 2,3,4,4'-Tetrahydroxy-BZP: 4.3×10^{-6} M (E2: 1.1×10^{-9} M; RBA: 0.025%) 2,2',4,4'-Tetrahydroxy-BZP: 1.4×10^{-6} M (E2: 1.3×10^{-9} M; RBA: 0.093%) 4-Chloro-4'-hydroxy-BZP: 1.9×10^{-5} M (E2: 1.6×10^{-9} M; RBA: 0.0081%) 4-Fluoro-4'-hydroxy-BZP: 4.8×10^{-5} M (E2: 1.1×10^{-9} M); 2.7×10^{-5} M (E2: 1.1×10^{-9} M); RBA: 0.0031% 2,3,4-Trihydroxy-BZP: 1.8×10^{-5} M (E2: 1.6×10^{-9} M; RBA: 0.0088%) 4,4'-Dibromo-BZP: 1.7×10^{-5} M (E2: 1.4×10^{-9} M; RBA: 0.0082%)	BZP derivatives (4-hydroxy/3-hydroxy/4,4'-dihydroxy/2,4-dihydroxy/2,4,4'-trihydroxy/2,3,4,4'-tetrahydroxy/2,2'4,4'-tetrahydroxy/4-chloro-4'-hydroxy/4-fluoro-4'-hydroxy/2,3,4-trihydroxy/4,4'-dibromo derivatives of BZP) have binding affinity for ER (the binding affinities were 1/1,100 - 1/44,000 of that of E2).	
		BZP IC50:>5X10-4M	No effect	Nakagawa & Tayama, 2001
		4-Hydroxy-BZP: 5×10^{-5} M	Binding affinity +	
Yeast two-hybrid assay	Cells: Yeast cells transfected with Gal4 DNA binding domain/human ER ligand binding domain genes, Gal4 activation domain/coactivator TIF2 genes and β-galactosidase reporter gene	REC10: $>3\times10^{-3}$ M (E2: 3×10^{-10} M)	BZP does not activate ER-mediated gene transcription.	Nishihara et al., 2000

Item	Test methods and conditions	Results	Conclusion	References
Reporter gene assay in yeast cells	Bioassay using yeast cells transfected with human estrogen receptor expression plasmid and estrogen receptor responsive element	BZP (1 μM) is negative for agonist activity. Some of the BZP derivatives have estrogen activity. The EC50 values for each of these derivatives are as shown below. 4-Hydroxy-BZP: 1.12×10^{-6} M 3-Hydroxy-BZP: 2.57×10^{-6} M 4-Amino-BZP:6.34×10^{-5} M 4,4'-Dihydroxy-BZP: 2.53×10^{-6} M 4,4'-Diamino-BZP: 5.89×10^{-5} M 4-Chloro-4'-hydroxy-BZP: 2.88×10^{-7}M 2,4-Dihydroxy-BZP: 2.4×10^{-6} M 2,3,4'-Trihydroxy-BZP: 5.08×10^{-6} M 2,4,4'-Trihydroxy-BZP: 5.64×10^{-7} M 2,2',4,4'-tetrahydroxy-BZP: 7.92×10^{-6} M	BZP does not activate ER-mediated gene transcription.	Schultz et al., 2000
	Bioassay using the yeast cells transfected with human progesterone receptor expression plasmid and progesterone receptor responsive element	BZP (1 μM) is negative for either agonist or antagonist activity.	BZP does not activate progesterone receptor-mediated gene transcription.	Tran et al., 1996
Reporter gene assay in recombinant cell cultures	Cells: HeLa cells transfected with human estrogen receptor expression plasmid and estrogen receptor responsive element. Exposure concentration: 10^{-11}-10^{-5} M	BZP is negative for agonist activity within a range of 10^{-11} - 10^{-5}M. Some of the BZP derivatives have the ability of gene transcription activation. The PC50[4)] values for each of these derivatives are as shown below. 4-Hydroxy-BZP: 2.6×10^{-6} M 3-Hydroxy-BZP: 2.6×10^{-6} M 4,4'-Dihydroxy-BZP: 1.6×10^{-6} M 2,4-Dihydroxy-BZP: 2.4×10^{-6} M 2,4,4'-Trihydroxy-BZP: 3.7×10^{-7} M 2,2',4,4'-Tetrahydroxy-BZP: 3.3×10^{-7} M 4-Chloro-4'-hydroxy-BZP: 1.8×10^{-6}M 4-Fluoro-4'-hydroxy-BZP: 2.0×10^{-6} M 4,4'-Dibromo-BZP: 2.7×10^{-6} M	BZP does not activate ER-mediated gene transcription.	CERI, 2001a
	Cells: HeLa cells transfected with rat ER expression gene and ER responsive element. Exposure concentration: 10^{-11} - 10^{-5}M	BZP is negative for agonist activity in the range of 10^{-11} - 10^{-5}M. (E2: PC50: $<10^{-9}$M)	BZP does not activate ER-mediated gene transcription.	Yamasaki et al., 2001
Human breast cancer cell proliferation assay	Bioassay using the proliferation of human breast cancer cells (MCF-7 cells) as the index.	4-Hydroxybenzophenone, a BZP derivative, has the proliferative activity (10-100 μM)(equivalent to 80% of 1 nM E2 at 100μM of 4-Hydroxybenzophenone).	BZP has no proliferative activity.	Nakagawa et al., 2000

ER: Estrogen receptor; E2: 17β-Estradiol; REC10: Concentration that produces activity equivalent to 10% of the activity of 10^{-7}M E2; PC50: Concentration that produces activity equivalent to 50% of the activity of 10^{-7}M E2.

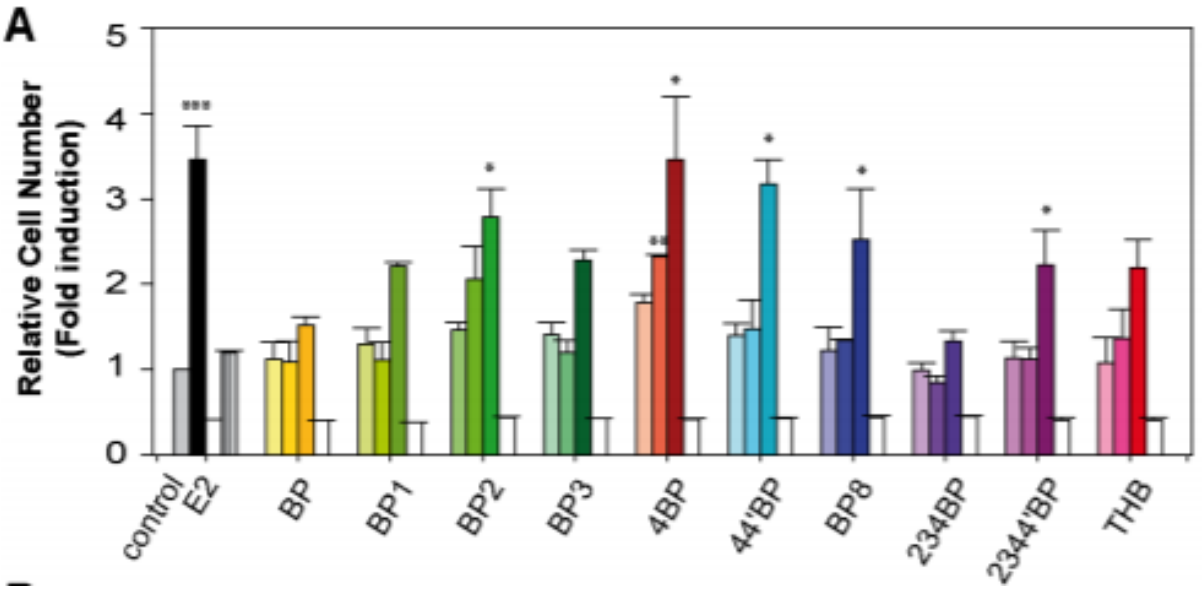


Figure 2. Proliferative effects of BPs in MCF-7 breast cancer cells. (**A**) After 48 h of steroid deprivation, MCF-7 cells were cultured in medium containing 2.5% dextran-treated charcoal stripped FBS and treated during 5 days with vehicle, 10^{-8} M estradiol (E2) or different concentrations of BPs (10^{-8}, 10^{-7} and 10^{-6} M). In addition, cells were treated with 10^{-7} M of the anti-estrogen $ICI_{182,780}$ (ICI) alone or in combination with 10^{-8} M E2 (hatched bar) or 10^{-6} M of each one of the BPs (open bars). Cell growth was evaluated using methylene blue assays and the results were expressed as fold induction between treated cells and vehicle-treated cells (considered as one-fold induction). (**B and C**) As in panel A, MCF-7 cells were

Kerdevil, 2013. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0060567

Recent findings on BP mutagenicity:

Evaluation by the Ames Assay of the Mutagenicity of UV Filters Using Benzophenone and Benzophenone-1
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6164588/

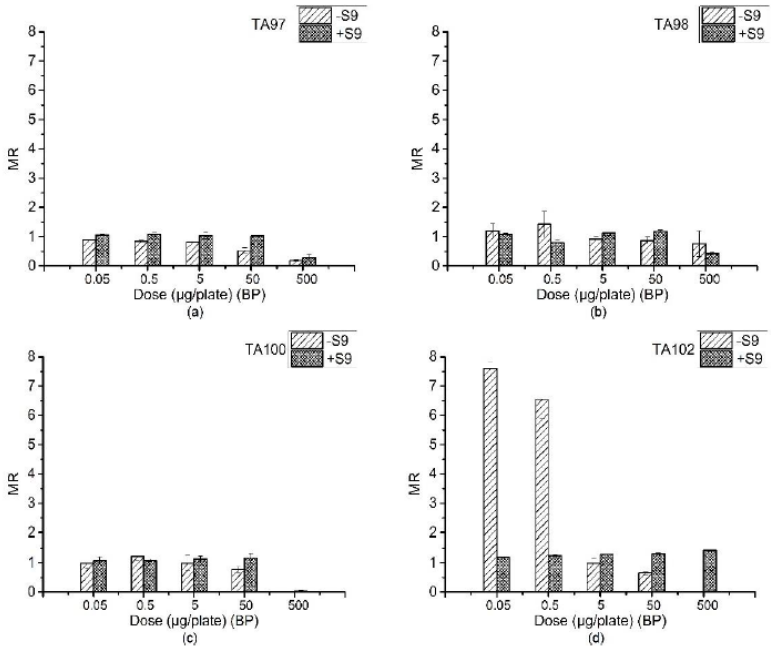


Figure 1. Mutagenesis of four strains by BP in the presence and absence of S9 liver extract; (a) TA97 strain; (b) TA98 strain; (c) TA100 strain; (d) TA102 strain. The mutagenicity ratio (MR) is the average ratio (±SE) from three parallel experiments.

Comparative toxicity related to metabolisms of benzophenone-type UV filters, potentially harmful to the environment and humans Mol Cell Toxicol (2017) 13:337-343

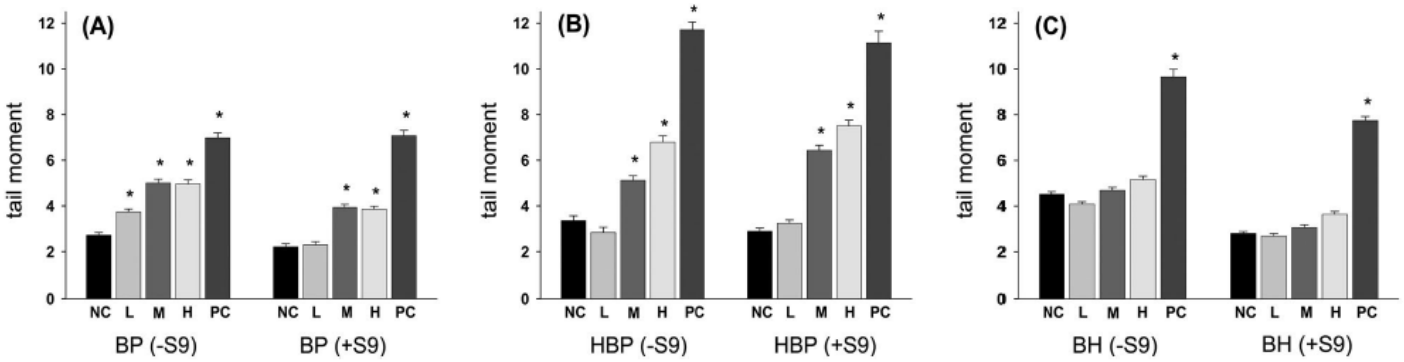


Figure 2. DNA damages by type A-UV filters, benzophenone (A), 4-hydroxybenzophenone (B) and benzhydrol (C), in L5178Y cells. Values are means ± SE from four experiments. In each experiment tail moment index had been assessed from 200 separately calculated cells. −S9=The absence of S9, +S9=The presence of S9, NC=Negative control (DMSO), PC=Positive control (−S9, MMS 150 μM, +S9, B[a]P 50 μM), Significance (*)=$P<0.05$.

Summary: the most established in vivo effect of BP are effects on the kidney and liver. The data from these studies are adequate to proceed with non-cancer and cancer hazards assessments. Mutagenicity findings for BPare negative but require additional review based on recent studies. In vitro endocrine activity for BP is negative but for 4-OH-BP is positive. Epidemiologic and animal studies do not support an endocrine effect for BP or 4-OH-BP however these studies are preliminary.