European Food Safety Authority

The EFSA Journal (2009) 1104, 1-30

SCIENTIFIC OPINION

Toxicological evaluation of benzophenone[1]

Scientific Opinion of the Panel on food contact materials, enzymes, flavourings and processing aids (CEF)

Question N° EFSA-Q-2009-411

Adopted on 14 May 2009

PANEL MEMBERS[*]

Arturo Anadón, David Bell, Mona-Lise Binderup, Wilfried Bursch, Laurence Castle, Riccardo Crebelli, Karl-Heinz Engel, Roland Franz, Nathalie Gontard, Thomas Haertlé, Trine Husøy, Klaus-Dieter Jany, Catherine Leclercq, Jean-Claude Lhuguenot, Wim Mennes, Maria Rosaria Milana, Karla Pfaff, Kettil Svensson, Fidel Toldrá, Rosemary Waring, Detlef Wölfle.

SUMMARY

Following a request from the European Commission to the European Food Safety Authority (EFSA), received on 19 February 2009, the Scientific Panel on food contact materials, enzymes, flavourings and processing aids (CEF) was asked:

A. to re-asses the TDI on benzophenone and hydroxybenzophenone in view of the new toxicological studies available, by 29 May 2009.
B. to evaluate if the substance 4-methylbenzophenone would be covered by the TDI on benzophenone and hydroxybenzophenone and evaluate the risk of the presence of 4-methylbenzophenone found in cereals by 3 March 2009.

---

[1] For citation purposes: Scientific Opinion of EFSA prepared by the Panel on food contact materials, enzymes, flavourings and processing aids (CEF) on Toxicological evalatuion of benzophenone. *The EFSA Journal* (2009) 1104, 1-30.

[*]D. Wölfle, M.R. Milana, K. Pfaff and K. Svensson declared that have advised their risk management Authorities on the issue. M.R. Milana declared that her Institute is conducting monitoring on benzophenone and 4-methylbenzophenone for the government. L. Castle declared that his laboratory had conducted the analytical work for the FSA survey report in 2000 and 2006 on benzophenone and 4-hydroxybenzophenone.
R. Franz declared that his Institute has performed analytical measurements on benzophenone and 4-methylbenzophenone for industries to test compliance of articles with the regulation.
In accordance with EFSA's Policy on Declarations of Interests and implementing documents thereof, and taking into account the specific matter discussed at the meeting, the interests above were not deemed to represent a conflict of interest for the experts concerned.

In the area of food packaging benzophenone and 4-methylbenzophenone are used as initiators for printing inks cured by UV radiation. Due to their volatility they can migrate through the packaging to the food if there is no functional barrier. No end uses were found in literature for 4-hydroxybenzophenone.

A. Based on the negative *in vitro* and *in vivo* results from tests with definite protocols the Panel concluded that benzophenone has no genotoxic potential.

Liver and kidney were identified as the primary target organs of benzophenone toxicity in rats and mice. The Panel considered neoplastic and non-neoplastic end-points in rats and in mice, obtained from chronic carcinogenicity and reproductive toxicity studies.

Benzophenone caused liver hypertrophy in the rat at the lowest dose level (~6 mg/kg/day) in a two-generation study, described as hypertrophy of centrilobular hepatocytes without any increase in liver weight. However, in a chronic carcinogenicity study in rats benzophenone did not cause liver tumours, even at exposure levels yielding severe liver damage. Therefore the Panel considers that the liver hypertrophy seen in rat is an adaptive response and not an adverse response. In the B6C3F1 mouse, benzophenone causes liver adenomas at a dose of 40 mg/kg b.w. per day. The Panel considers the liver adenomas to be an adverse effect. However, this effect is a less sensitive endpoint than the kidney effects.

The Panel noted that benzophenone causes kidney adenoma in rat, associated with a spectrum of responses including hyperplasia and nephropathy at the lowest dose level of 15 mg/kg/day in a chronic carcinogenicity study. The Panel considered that the non-neoplastic kidney effects observed in the chronic assay were adverse. Benchmark dose (BMD) analyses were applied for the non-neoplastic kidney effects in male rats, and the lower 95% confidence limits of the bench mark dose for a 10% effect (BMDL10) were calculated to be 3.1 to 7.4 mg/kg b.w. per day. The models used in the analysis were consistent, and passed statistical validation. The Panel decided that the BMDL10 value of 3.1 mg/kg b.w. per day was the most appropriate departure point for derivation of the TDI. By applying an uncertainty factor of 100, a TDI of 0.03 mg/kg body weight is derived.

The Panel noted that the SCF has established for benzophenone and 4-hydroxybenzophenone a group TDI of 0.01 mg/kg body weight. The evaluation of the SCF was only based on a metabolism study and on a 90-day oral rat study on benzophenone.

4-Hydroxybenzophenone is one of the two important metabolites of benzophenone. However, the Panel considers that this fact alone, in the absence of supporting data, does not justify the inclusion of 4-hydroxybenzophenone in the same TDI with benzophenone.

B. For the purpose of providing an urgent advice on 4-methylbenzophenone to the risk managers, the EFSA in its statement, issued on 4 March 2009, considered the liver hypertrophy seen in the two-generation study with benzophenone as adverse effect. Thus, EFSA derived a LOAEL of 6 mg benzophenone/kg b.w. per day from this study.
This LOAEL was used as a basis for calculation of the Margin of Exposure (MoE) for 4-methylbenzophenone. A factor of 100 for inter- and intraspecies differences in sensitivity, a factor of 3 for use of a LOAEL (instead of a NOAEL) and a factor of 2 for read-across from benzophenone to 4-methylbenzophenone were applied. Hence the estimated MoE should be greater than 600. The MoE is calculated by dividing the LOAEL (6 mg/kg b.w. per day) by the estimated exposure in each case.

In the EFSA statement dietary exposure to 4-methylbenzophenone was calculated for adults and children from breakfast cereals and from all foods, based on levels of 4-methylbenzophenone in a limited number of breakfast cereals samples and on levels of benzophenone in other foods as recorded in the British Food Standards Agency surveys; benzophenone was used as a proxy for 4-methylbenzophenone, given that these two substances are used for a similar purpose.

The EFSA concluded that for adults the estimated dietary exposure is unlikely to lead to a health concern. For children, the estimated exposure based on a conservative scenario (high consumption of breakfast cereals, average concentration of 4-methylbenzophenone) was also unlikely to pose a health concern. However, for children, based on the highly conservative scenario (high consumption of breakfast cereals, highest concentration of 4-methylbenzophenone), a health concern could not be excluded.
The full Statement is available through: http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902360964.htm

For the current opinion the Panel after having examined the toxicological data on benzophenone considers that the liver hypertrophy seen in rat in the two-generation study is an adaptive response and not an adverse response. Therefore the Panel considered the EFSA approach to derive a LOAEL of 6 mg/kg b.w. per day based on this effect as conservative. Due to the limited amount of time available to EFSA for the release of its statement the Panel considered this conservative approach reasonable.

The Panel derives a TDI of 0.03 mg/kg b.w. per day for benzophenone based on the BMDL10 value of 3.1 mg/kg b.w. per day following BMD analyses on the non-neoplastic kidney effects in male rats. This value is proposed to be used as a basis for calculation of the Margin of Exposure (MoE) for 4-methylbenzophenone.
A factor of 100 for inter- and intraspecies differences in sensitivity and a factor of 2 for read-across from benzophenone to 4-methylbenzophenone are applied. Hence the estimated MoE should be greater than 200. The MoE is calculated by dividing the BMDL10 (3.1 mg/kg b.w. per day) by the estimated exposure in each case.

According to a highly conservative scenario as described in the EFSA statement (high consumption of breakfast cereals – highest concentration of 4-methylbenzophenone + assumptions for other foods) the highest dietary exposure to 4-methylbenzophenone is 15.2 µg/kg b.w. per day for children.
The MoE for this exposure estimate as calculated against the BMDL10 value for benzophenone in this opinion is 204. (3100 divided by 15.2)
This MoE is not less that the minimum required MoE of 200.

The Panel concluded that short term consumption of contaminated breakfast cereals at current levels should not pose a risk to people. This conclusion is based on the limited exposure data available and read across from the toxicity of the similar substance benzophenone. If the use of 4-methylbenzophenone is to be continued, more data on occurrence of the substance in foods should be provided as well as appropriate toxicity data corresponding to the level of exposure for a full risk assessment.

Key words: benzophenone, 4-hydroxybenzophenone, 4-methylbenzophenone, UV photoiniators, printing inks, cardboard, 119-61-9, 1137-42-4, 134-84-9.

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY THE COMMISSION

A recent notification from the German authorities under Art 50 of the Regulation (EC) No178/2002 on the *Rapid Alert System for Food and Feed* (RASFF) has reported the migration of 4-methylbenzophenone from packaging into certain cereal products in a quantity of 798 micrograms/kg. According to the German authorities the contamination derives from the transfer of 4-methylbenzophenone from the printed surface of the cardboard box where it is used as photo-initiator in the UV hardened lacquer.

Inks are not covered by a specific European legislation on food contact materials. However, the use of printing inks has to comply with the general rules of Regulation (EC) No 1935/2004 and with good manufacturing practice as laid down in Commission Regulation (EC) No 2023/2006.

The Belgian authorities reported later that during storage the migration can be as high as 3729 µg/kg. Based on this migration value, the Belgian authorities have provided to the Commission a draft risk assessment which is sent to EFSA with this request.

On behalf of the printing ink producers and of the cardboard box manufacturers, the European Printing Ink Association (EuPIA) has provided risk assessments performed on their request. These are also sent to EFSA with this request.

The above two mentioned risk assessments apply a read across approach using toxicological data available for benzophenone and hydroxybenzophenone. The Scientific Committee on Food has established in 1991 and 1992 for the related substances hydroxybenzophenone and benzophenone a tolerable daily intake (TDI) of 0.01 mg/k bodyweight (b.w.) (SCF 1991; SCF, 1992). From the provided risk assessments it becomes evident that new toxicological data are available on benzophenone since the establishment of the TDI which may influence the TDI.

TERMS OF REFERENCE AS PROVIDED BY THE COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Commission (Letter from the Commission on 18.02.2009 received on 19.02.2009) asks the European Food Safety Authority to evaluate if the substance 4-methylbenzophenone would be covered by the TDI on benzophenone and hydroxybenzophenone and evaluate the risk of the presence in of 4-methylbenzophenone found in cereals by 3 March 2009. In addition the Commission asked EFSA to re-assess the TDI on benzophenone and hydroxybenzophenone in view of the new toxicological studies available, by end of May 2009.

PROPOSED ACTION TO THE TERMS OF REFERENCE

In relation to the Commission request to evaluate if the substance 4-methylbenzophenone would be covered by the TDI on benzophenone and hydroxybenzophenone, as established by the Scientific Committee on Food, and to evaluate the risk of the presence of 4-methylbenzophenone found in cereals, EFSA was only able to publish an EFSA statement on this issue rather than a scientific opinion of the CEF Panel due to the very short deadline (EFSA, 2009)

In addition to this EFSA statement, the CEF Panel adopts this opinion concerning the request to re-assess the group TDI on benzophenone and hydroxybenzophenone in view of the new toxicological studies available (Letter of the Executive Director of EFSA to the Commission on 20.02.2009). The Panel is also commenting on the conclusions of the EFSA statement in view of the toxicological assessment of benzophenone.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the food contact materials working group, Mona-Lise Binderup, Laurence Castle, Riccardo Crebelli, Roland Franz, Nathalie Gontard, Eugenia Lampi, Jean-Claude Lhuguenot, Maria Rosaria Milana, Karla Pfaff, Kettil Svensson, Detlef Wölfle for their input in drafting of this opinion and Salomon Sand for performing the BMD modeling.

EVALUATION

1. Introduction

In the area of food packaging, benzophenone is widely used as photoinitiator for inks and lacquers that are cured with ultraviolet light. UV-cure inks and lacquers are used without solvent and they contain typically 5–10% photoinitiator. UV-cure lacquers are commonly employed either as varnishes for UV-cure printing or as varnishes for materials printed by other processes. Benzophenone is not completely used up or removed during or after the printing process, nor is it bound irreversibly into the print film layer (Anderson and Castle, 2003).

Benzophenone may also be present in recycled printed paper or board if the recycling process has not fully stripped the substance from the recovered paper.

Due to its volatility benzophenone may migrate through paperboard to the food if no effective barrier is present. Internal plastic bags, used a barrier to moisture, do not always act as an effective barrier (Song *et al*, 2003, Choi *et al*, 2002, Feigenbaum *et al*, 2005, Pastorelli *et al*, 2008). Specific migration studies have shown that benzophenone can migrate from paper and board into dry foods (Triantafyllou *et al*, 2007, Jickells *et al*, 2005, Anderson and Castle, 2003, Johns *et al*, 2000) or powders simulating dry foods (Nerin and Assensio, 2002).

Benzophenone might also find use as a photoinitiator for copolymerisation or crosslinking of polyolefins (Felisberti, M.-I. *et al.*, 1985; Lei, J. and Liao, X., 2000).

Benzophenone is also used as a food flavouring. In its survey report on benzophenone migration from food packaging to foodstuff, the UK Foods Standard Agency (FSA) noted that "there may have been some contribution to benzophenone levels in some samples from its use as a flavouring" (FSA, 2000).

Benzophenone is also used in fragrances, cosmetics (UV filters in sun creams), toiletries, pharmaceuticals and insecticides. Exposure from all these sources may be significant (NTP, 2006).

Benzophenone is included in the list of additives authorised for food contact plastics and it has a specific migration limit of 0.6 mg/kg food (EC, 2002) following an opinion of the Scientific Committee on Food in 1992 which established a group TDI for benzophenone and 4-hydroxybenzophenone of 0.01 mg/kg b.w. (SCF, 1992). The evaluation of the SCF was only based on a metabolism study and on a 90-day oral rat study on benzophenone.

The CEF Panel based its evaluation on toxicological studies on benzophenone published after the adoption of the SCF opinion in 1992. No toxicological studies on 4-hydroxybenzophenone were found in the literature.

2. Legislation

Printing inks are not covered by specific European legislation on food contact materials. However the use of printing inks has to comply with the general rules of Regulation (EC) No 1935/2004 and with good manufacturing practice (GMP) as laid down in Commission Regulation (EC) No 2023/2006.

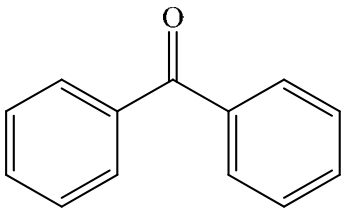
In relation to components of packaging such as inks, Regulation (EC) No 1935/2004 states that under normal or foreseeable conditions of use they should not transfer their constituents to food in quantities which could endanger human health.

There is a specific migration limit (SML) for benzophenone of 0.6 mg/kg (Directive 2002/72/EC) for its use as an additive in plastics following an opinion of the Scientific Committee on Food in 1992 which established a group TDI for benzophenone and 4-hydroxybenzophenone of 0.01 mg/kg b.w. (SCF, 1992). The evaluation of the SCF was based on a metabolism study and on a 90-day oral rat study.

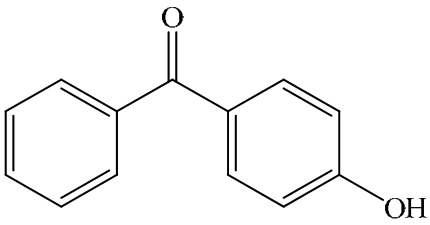
3. Chemistry

Benzophenone (CAS No. 119-61-9) and 4-hydroxybenzophenone (CAS No. 1137-42-4) are aromatic ketones with a molecular weight of 182 g/mol and 198 g/mol respectively. 4-Hydroxybenzophenone differs from benzophenone only by a hydroxyl group. Given their conjugated structure, with a carbonyl group bridging two phenyl rings, they are strong absorbers of UV light. Consequently, they may find use in sun screen creams and as photoinitiators for polymerisation reactions.

Benzophenone is sparingly soluble in water (32 mg/L) and freely soluble in organic solvents such as ethanol, benzene and propylene glycol. It is lipophilic with an octanol/water partition coefficient (LogPo/w) of 3.2. It is semi volatile with a discernable vapour pressure even at room temperature (vapour pressure 1.2 Pa at 25°C).
4-Hydroxybenzophenone has similar values of Log Po/w and vapour pressure.



Benzophenone



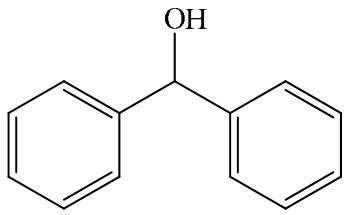
4-Hydroxybenzophenone

4. Toxicity

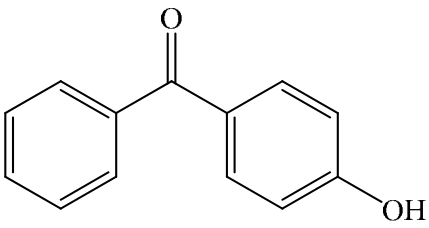
4.1. Metabolism of benzophenone

In vitro, benzophenone is metabolised to benzhydrol (reduction of ketone group), 4-hydroxybenzophenone (aromatic hydroxylation) (Figure 1) and to the sulphate conjugate (Nakagawa *et al*, 2000). Studies *in vivo* in rats show that, as in the *in vitro* reports, benzophenone mainly gives benzhydrol and 4-hydroxybenzophenone (Nakagawa and Tayama, 2002; Jeon *et al.*, 2008) probably with the sulphate and glucuronide conjugates which may undergo enterohepatic circulation. Benzhydrol glucuronide is likely to be a major urinary metabolite and a substrate of the organic anion transporters in the kidney (NTP, 2006; Buist and Klaassen, 2004).

Figure 1: Important metabolites of benzophenone (according to Nakagawa *et al.*, 2000 and Jeon *et al.*, 2008)



Benzhydrol



4-Hydroxybenzophenone

4.2. Genotoxicity of benzophenone

Benzophenone has been extensively tested in the framework of an NTP research programme (NTP, 2000 and 2006). Benzophenone (1-1000 µg/plate) was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537, with or without hamster or rat liver activation enzymes. Benzophenone and its metabolites benzhydrol and p-benzoylphenol induced *umu* gene expression, which may be indicative of DNA damage, in *S.typhimurium* TA1535/pSK1002 when tested in presence of recombinant human P450s expressed on *E.coli* membranes (Takemoto *et al*., 2002); in the same study negative results were obtained with human, rat or mouse microsomes, as well as with human liver S9 and cytosolic fractions, suggesting the detoxication of P450-mediated metabolites by other enzymes present in liver preparations. Overall, the relevance for genotoxicity assessment of the positive findings reported by Takemoto *et al.* is limited in view of the indirect link between *umu* expression and mutagenicity, and of the artificial metabolic activation system required.
Negative results were reported with benzophenone in a mammalian mutation assay with mouse lymphoma L5178Y/tk+/- cells (CCRIS, 1991). In male B6C3F1 mice, intraperitoneal injections of 200 to 500 mg benzophenone/kg body weight (three injections at 24-hour intervals) did not induce a statistically significant increase in the frequencies of micronuclei in bone marrow PCEs. In addition, no increases in micronucleated normochromatic erythrocytes were noted in peripheral blood of male or female mice administered benzophenone for 14 weeks in dosed feed over a concentration range of 1250 to 20000 ppm (NTP, 2006). Based on experimental evidence available, the Panel concluded that benzophenone has no genotoxic potential.

4.3. Repeated-dose studies on benzophenone

Groups of male rats (strain not specified) were fed diets containing 0.1% or 1.0% benzophenone for 10 consecutive days (USEPA, 1984). Feed consumption and body weights were slightly reduced in the 1.0% group. Exposure concentration-dependent increases in absolute and relative liver weights and relative kidney weight were observed. Serum alanine aminotransferase activity of rats in the 1.0% group was increased compared to that of the controls. Mild degenerative effects were observed in the liver and bone marrow of rats in the 1.0% group, suggesting that the liver may be the primary target of the toxic effects of benzophenone and that the bone marrow may also be a target.

Benzophenone was administered in the diet to rats at target dose levels of 20 mg/kg body weight/day for 90 days and 100 or 500 mg/kg body weight/day for 28 days (Burdock *et al.*, 1991). Treatment-related changes occurred in erythrocyte count, haemoglobin, haematocrit, bilirubin, total protein and albumin at the mid- and high-dose levels, although all changes did not occur in both groups in both sexes. There were indications of increased absolute and relative liver and kidney weights in the mid- and high-dose groups, but this was not statistically consistent for absolute kidney weights. Histopathology of the liver in the mid- and high-dose groups showed hepatocellular enlargement with an associated clumping of cytoplasmic basophilic material around the central vein. A no-effect level was demonstrated at 20 mg/kg body weight/day for 90 days of administration.

Fourteen-week studies with benzophenone were performed in rats and mice (NTP, 2000). Rats were treated with daily doses ranging from 75 to 850 mg/kg body weight for males and from 80 to 1000 mg/kg body weight for females; doses from 200 to 3300 mg/kg body weight were given to male mice and from 270 to 4200 mg/kg body weight to female mice. Increases in liver weights were attributed to centrilobular hypertrophy (in mice in all treatment with dose-dependent increases in severity) and cytoplasmic vacuolization of hepatocytes (in all examined male rats from all treatment groups). Based on these findings no NOAEL could be derived from the studies in rats and mice.
In rats exposure-related increases in alanine aminotransferase and bile salt concentrations indicated a hepatic effect consistent with the gross and microscopic liver changes. These alterations were accompanied by benzophenone-induced increases in pentoxyresorufin dealkylase, an enzyme activity linked to the cytochrome P450 2B isomer. In rats, the kidney was also identified as an additional target organ based on increases in kidney weights and microscopic changes including tubule dilatation (in all treated males – except the lowest dose group; in the two highest dose groups in females), protein casts, tubule epithelial regeneration, mineralization, and necrosis in renal papillae. Unique lesions were well-demarcated, wedge-shaped areas of prominent tubule dilatation. Additionally, the rat hematopoietic system was affected by benzophenone (mild anemia, altered circulating erythroid mass, and transient exposure concentration-related increases in platelet counts).
In mice, increased liver weights (up to 100% in males and 62% in females) were associated with increased incidences of centrilobular hypertrophy of hepatocytes in all exposed groups. The severity of hepatocyte hypertrophy increased in an exposure concentration-related manner,with moderate to marked severity in all high dose groups of mice mice. Male mice exhibited evidence of anemia, demonstrated by minimal decreases in hematocrit values, hemoglobin concentrations, and erythrocyte counts. As in rats, kidney weights were increased in all exposed animals, except in the low dose group. However, unlike in rats, there were no microscopic effects to account for the increased weights.

4.4. Carcinogenicity studies on benzophenone

In recent 2-year studies (NTP 2006) liver, kidney, nose and testes were identified as target organs in F344/N rats and B6C3 F1 mice. Neoplastic responses occurred in the kidney, liver and hematopoietic system. Some evidence for carcinogenic activity of benzophenone was reported in male rats based on increased incidences of renal tubule adenoma and evidence in female rats was equivocal based on the marginal increased incidences of mononuclear cell leukemia and histiocytic sarcoma. In mice, there was some evidence of carcinogenic activity of benzophenone based on increased incidences of hepatocellular neoplasms and increased incidences of histiocytic sarcoma in males and females, respectively. There was equivocal

evidence of carcinogenic activity of benzophenone in female rats based on the marginal increased incidences of mononuclear cell leukemia and histiocytic sarcoma.
Groups of 50 male and 50 female F344/N rats and B6C3 F1 mice were fed diets containing 0, 312, 625, and 1250 ppm benzophenone for 105 weeks. This corresponds to average daily doses of approximately 15, 30, and 60 mg benzophenone/kg body weight per day to male and 15, 30, and 65 mg/kg body weight/day to female rats; for mice the corresponding doses are 40, 80, and 160 mg benzophenone/kg body weight/day to males and 35, 70, and 150 mg/kg body weight to females. Animals were observed twice daily for moribundity and mortality. Body weights and clinical findings were recorded at 4-week intervals. Necropsies and microscopic examinations were performed on all animals.
Body weight reductions were observed at 30 and 60/65 mg/kg body weight per day in male and female rats. In male rats, an increased mortality in the high dose group (after 80 weeks) was partly attributed to nephropathy caused by benzophenone exposure. There was also a positive trend in the incidence of renal tubule adenoma in male rats; these neoplasms were accompanied by significantly increased incidences of renal tubule hyperplasia and a dose-dependent enhancement of the severity of nephropathy in all treatment groups (Table 1). In females a significantly enhanced severity of nephropathy was found at 30 and 65 mg/kg body weight per day.

Table 1: Kidney and liver effects in male F344/N rats (2-year study: NTP, 2006)

Conc. in feed **Doses**	0, 312, 625, 1250 ppm 0, 15, 30, 60 mg/kg body weight per day
Non- **neoplastic effects**	**Kidney:** renal tubule, hyperplasia (standard evaluation :1/50, 5/50, 20/50, 23/50; standard / 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)
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)

Liver lesions observed included significantly increased incidences of hepatocytic centrilobular hypertrophy in all exposed groups of rats. Bile duct hyperplasia was increased in all treated females. Mononuclear cell leukemia was observed in all groups of rats, but with increased incidences in benzophenone exposed groups. Fibroadenomas were dose-dependently decreased in female rats.

In mice, survival of all exposed groups was generally similar to that of the control groups except in high dose females (14% less than controls). Reduced body weights were observed in treated female mice, but not in males. In male mice, there were significantly increased incidences of hepatocellular adenoma in the mid and high dose groups (22%, 30%, 46% and 46% at 0, 40, 80, 160 mg/kg body weight per day, respectively); the number of male animals with multiple adenoma was significantly increased in all treatment groups (2, 8, 8 and 12 at 0, 40, 80, and 160 mg/kg body weight per day, respectively). In female mice, the incidences of hepatocellular adenoma in the mid and high dose groups (70 and 150 mg/kg body weight per day) were higher than expected after adjusting for the lower body weights in these groups. Mild to moderate hyperplasia in centrilobular hepatocytes was observed in all treatment groups. Additionally, increased incidences of a number of liver lesions were found in treated male mice (clear cell foci, multinucleated hepatocytes, necrosis, chronic active inflammation and cystic degeneration). The incidences of hepatocellular carcinomas were similar in all groups. The incidences of nephropathy in exposed groups of female mice, as well as the severity of nephropathy in exposed groups of males, were significantly increased. The incidences of metaplasia of the olfactory epithelium were significantly increased in mice at 160/150 mg/kg body weight per day. Effects in the spleen were increased haematopoietic cell proliferation in females and hyperplastic changes in all treated mice. In male mice an increased mineralization in the testes was reported. Rare histiocytic sarcomas were observed in female rats and mice in the mid and high dose groups (70 and 150 mg/kg body weight per day).
No NOAELs could be derived from these studies. The LOAEL for the rat study was 15 mg/kg body weight per day based on increased incidences of mononuclear cell leukaemia and bile duct hyperplasia in all treated females and nephropathy and renal tubule hyperplasia in all treated males. The LOAEL for mice was ~35mg/kg body weight per day based on multiple hepatocellular adenoma in treated males and nephropathy accompanied by mineralization in treated females and increased severity of nephropathy in treated males.

Additionally, dermal studies on carcinogenicity of benzophenone have been performed with female Swiss mice (Stenbäck and Shubik, 1974) and New Zealand White rabbits (Stenbäck, 1977). In lifetime studies, animals received twice-weekly topical administrations of 0.02 mL of 5%, 25%, or 50% benzophenone in acetone. Benzophenone was applied to a 1-inch square area on the dorsal skin between the flanks of mice; for rabbits, the dose was applied to the inside of the left ear. All mice died by week 110. The incidences of skin neoplasms in dosed mice were similar to those in the controls (Stenbäck and Shubik, 1974). Benzophenone had no effect on survival rates or on the incidences of neoplasms or nonneoplastic lesions in rabbits after 160 weeks of treatment (Stenbäck, 1977).

The negative results obtained with benzophenone in carcinogenicity studies by dermal application are in line with the presumed non-genotoxic mode of action of this compound, in view of the prevailing occurrence of genotoxic carcinogens among those active by topical application.

4.5. Reproductive and developmental toxicity studies on benzophenone

In a developmental toxicity study, benzophenone was administered by gavage to timed pregnant Sprague-Dawley (CD®) rats (22 to 25 per group) at doses of 100, 200, or 300 mg/kg body weight per day on days 6 to 19 of gestation (NTP, 2002). While there were no treatment-related maternal deaths, maternal toxicity occurred in all dosed groups. Clinical signs observed at all doses of benzophenone included lethargy, piloerection, weight loss, and

rooting in bedding after dosing. Maternal liver and kidney weights were significantly increased in all dosed groups. Reduced maternal body weight gain and decreased feed consumption were observed in the 300 mg/kg group. Benzophenone had no adverse effects on prenatal viability or overall incidences of fetal malformations or variations. However, average fetal body weight per litter in the 300 mg/kg group was significantly lower than that in the vehicle controls. The incidences of unossified sternebrae were increased at all doses of benzophenone, and the incidences of extra rib on Lumbar I were increased in the 200 and 300 mg/kg groups. Although a NOAEL was not achieved for developmental toxicity, the effects described above are limited to mild developmental delays with a high probability of recovery during early postnatal development.

In another study, New Zealand White rabbits (24 per group) were administered benzophenone by oral gavage at 5, 25, or 45 mg/kg body weight on gestational days 6 to 29 (NTP, 2004a). Maternal body weight and feed consumption showed decreasing trends. There were no changes in the weights of the gravid uterus, liver, or kidney of treated does. Benzophenone had no significant adverse effect on prenatal viability in litters that were carried until scheduled termination on gestational day 30. Nevertheless, the ability of does to successfully carry their pregnancies was clearly compromised in a dose related manner (0 mg/kg, 24/24; 5 mg/kg, 24/24; 25 mg/kg, 19/22; 45 mg/kg, 12/19). Fetal body weight was significantly decreased in the 45 mg/kg group only. Similar to the rat studies, developmental toxicity was noted only in the presence of well-defined maternal toxicity. Thus, there was no evidence for selective susceptibility of the conceptus relative to the pregnant dam in either the rat or the rabbit.

The reproductive toxicity of benzophenone was examined in a two-generation study in rats (Hoshino *et al*, 2005). Male and female Sprague-Dawley (SD) rats, parental (F0) and first generation (F1), were exposed to benzophenone by feeding diet with concentrations of 0 (control), 100, 450 or 2000 ppm (corresponding approximately to doses of 6-9, 29-40 and 130-179 mg/kg body weight/day, respectively) . In F0 and F1 parental animals, inhibition of body weight gain and food consumption, significantly elevated renal weights, dilatation of the renal proximal tubules, and regeneration of the proximal tubular epithelium were recognized at doses of 450 ppm and 2000 ppm, along with an increase in hepatic weight and centrilobular hepatocytic hypertrophy (Table 2). Obvious effects on the endocrine system and reproductive toxicological effects were not observed up to the highest dose of 2000 ppm in the F0 or F1 parent animals (no test substance related changes in the estrous cycle, reproductive capability, delivery and lactation, sperm parameters, serum hormone levels, or necropsy findings). As for effects on the offspring, inhibition of body weight gain was observed in both the F1 and F2 males and females of the 2000 ppm group, but no other treatment-related effects were observed (in the number of male and female F1 or F2 pups delivered, viability, anogenital distance, physical development, the results of reflex and response tests, or on the observation results of external abnormalities)., where there was an increase in liver weight and centrilobular hypertrophy, and in kidney. Based on the dose-dependent histopathological findings in liver of adult rats a LOAEL of 6 mg/kg body weight per day was derived. Reproductive toxicity was not observed in this study, effects on the offspring were observed at the highest dose only.

Table 2: Hypertrophy of centrilobular hepatocytes in rats (Hoshino *et al.*, 2005)

Conc. in feed (ppm)	**0**	**100**	**450**	**2000**
F0, males (mg/kg b.w. per day)	-	6.4	29.0	130.0
No. examined	24	23	24	24
Effect +	0	9	24	0
++	0	0	0	24
F0, females (mg/kg b.w. per day)*	-	8.4	38.2	166.5
No. examined	24	24	24	24
Effect +	0	4	21	0
++	0	0	0	24
F1, males (mg/kg b.w. per day)	-	7.8	34.6	159.4
No. examined	22	22	23	24
Effect +	0	9	22	4
++	0	0	1	20
F1, females (mg/kg b.w. per day)*	-	8.7	40.5	179.2
No. examined	23	22	23	23
Effect +	0	7	23	0
++	0	0	0	23

* Mean daily intake (pre-mating + gestation + lactation periods)
+ Slight, ++ Moderate

4.6. Estrogenic effects of benzophenone

Immature, 21-day-old female Sprague-Dawley rats (unspecified number of animals per group) were used to compare uterotrophic effects of 17 β-estradiol, benzophenone, and two metabolites of benzophenone, *p*-hydroxybenzophenone and benzhydrol (Nakagawa and

Tayama, 2001). Animals were dosed with 100, 200, or 400 mg benzophenone, *p*-hydroxybenzophenone, or benzhydrol per kg body weight via subcutaneous injection once per day for 3 days and sacrificed 6 hours after the last dose. Neither benzophenone nor benzhydrol affected uterine weight or morphology of the uterus or vagina. *p*-Hydroxy-benzophenone elicited increases in absolute and relative uterine weights in a dose-dependent manner and increased the luminal epithelium height and thickness of the stromal layer of the uterus at 400 mg/kg. In the vagina, *p*-hydroxybenzophenone increased the thickness of the epithelial cell layer, accompanied by cornification. A subsequent study examined the effects of benzophenone on ovariectomized rats (Nakagawa and Tayama, 2002). Female Sprague-Dawley rats (five per group) were ovariectomized at 4 weeks of age, acclimated for 3 weeks, orally administered 100 or 400 mg/kg body weight for 3 days, and sacrificed 24 hours after the last dose. The 400 mg/kg dose of benzophenone elicited approximately 1.9-fold increases in absolute and relative uterine weights. The uterine response was accompanied by increased luminal epithelium height and thickness of the stromal layer of the uterus. Additionally, benzophenone (400 mg/kg) increased the thickness of the vaginal epithelial cell layers with cornification.

Benzophenone and various derivatives were also tested in different *in vitro* assays to determine their estrogenic and anti-androgenic activities. Benzophenone neither induced the estrogen responsive element-dependent reporter gene transcription (Schultz *et al.*, 2000) nor the estrogen-dependent proliferation of the human breast cancer cell line MCF-7 (Nakagawa *et al.*, 2000). Benzophenone and benzhydrol did not bind to the human estrogen receptor alpha (Nakagawa and Tayama, 2001). In contrast, hydroxylated benzophenones exhibited estrogenic activity in MCF-7 cells (Suzuki *et al.* 2005), bound the human estrogen receptors alpha and beta and were active in human estrogen receptor-specific assays (Molina-Molina *et al.*, 2008), but their activities varied markedly depending on the degree of hydroxylation. Benzophenone and some related compounds showed significant inhibitory effects on the androgenic activity in responsive cell lines (Suzuki *et al.* 2005; Molina-Molina *et al*., 2008).

In addition, benzophenone was shown to induce the expression of cytochrome P450-dependent activities, including CYP3A1 via a pregnane X receptor-mediated mechanism and thus may influence hormonal homeostasis (Mikamo *et al.*, 2003).

4.7. Derivation of a TDI

Liver and kidney were identified as the primary target organs of benzophenone toxicity in rats and mice. The Panel considered neoplastic and non-neoplastic end-points in rats and in mice, obtained from chronic carcinogenicity (NTP, 2006) and reproductive toxicity studies (Hoshino *et al.*, 2005).

Benzophenone caused liver hypertrophy in the rat at the lowest dose level (~6 mg/kg/day) in a two-generation study (Hoshino *et al.*, 2005), described as a slight hypertrophy of centrilobular hepatocytes without any increase in liver weight. However, in a chronic carcinogenicity study in rats benzophenone did not cause liver tumours, even at exposure levels yielding severe liver damage. Therefore the Panel considers that the liver hypertrophy seen in rat is an adaptive response and not an adverse response. In the B6C3F1 mouse, benzophenone causes liver adenomas at a dose of 40 mg/kg/day. The Panel considers the liver adenomas to be an adverse effect. However, this effect is a less sensitive endpoint than the kidney effects.

The Panel noted that benzophenone causes kidney adenoma in rat, associated with a spectrum of responses including hyperplasia and nephropathy at the lowest dose level of 15 mg/kg/day in a chronic carcinogenicity study (NTP, 2006). The Panel considered that the non-neoplastic kidney effects observed in the chronic assay were adverse. Benchmark dose (BMD) analyses were applied for the non-neoplastic kidney effects in male rats (NTP, 2006), and the lower 95% confidence limits of the bench mark dose for a 10% effect (BMDL10) were calculated to be 3.1 to 7.4 mg/kg b.w. per day (Appendix). The models used in the analysis were consistent, and passed statistical validation. The Panel decided that the BMDL10 value of 3.1 mg/kg b.w. per day was the most appropriate departure point for derivation of the TDI. By applying an uncertainty factor of 100, a TDI of 0.03 mg/kg body weight is derived.

The Panel noted that the SCF has established for benzophenone and 4-hydroxybenzophenone a group TDI of 0.01 mg/kg body weight (SCF, 1992). The evaluation of the SCF was only based on a metabolism study and on a 90-day oral rat study on benzophenone.

4-Hydroxybenzophenone is one of the two important metabolites of benzophenone. However, the Panel considers that this fact alone, in the absence of supporting data, does not justify the inclusion of 4-hydroxybenzophenone in the same TDI with benzophenone.

5. Discussion on 4-methylbenzophenone - Consequences of the derivation of a new TDI for benzophenone

The EFSA has issued an EFSA Statement on 4 March 2009 (EFSA, 2009) responding to an urgent request of the European Commission if the substance 4-methylbenzophenone would be covered by the TDI on benzophenone and hydroxybenzophenone and to evaluate the risk of the presence of 4-methylbenzophenone found in cereals.
For the purpose of providing an urgent advice to the risk managers, the EFSA in its statement considered the liver hypertrophy seen in the two-generation study (Hoshino *et al.*, 2005) with benzophenone as adverse effects. Thus, EFSA derived a LOAEL of 6 mg benzophenone/kg b.w. per day from this study.
This LOAEL was used as a basis for calculation of the Margin of Exposure (MoE) for 4-methylbenzophenone. A factor of 100 for inter- and intraspecies differences in sensitivity, a factor of 3 for use of a LOAEL (instead of a NOAEL) and a factor of 2 for read-across from benzophenone to 4-methylbenzophenone were applied. Hence the estimated MoE should be greater than 600. The MoE is calculated by dividing the LOAEL (6 mg/kg b.w. per day) by the estimated exposure in each case.

In the EFSA statement dietary exposure to 4-methylbenzophenone was calculated for adults and children from breakfast cereals and from all foods based on levels of 4-methylbenzophenone in a limited number of breakfast cereals and on levels of benzophenone in other foods as recorded in the British Food Standards Agency surveys (FSA, 2000 and 2006), as a proxy for 4-methylbenzophenone, given that these two substances are used for a similar purpose.
The EFSA concluded that for adults the estimated dietary exposure is unlikely to lead to a health concern. For children, the estimated exposure based on a conservative scenario (high consumption of breakfast cereals, average concentration of 4-methylbenzophenone) was also unlikely to pose a health concern. However, for children, based on the highly conservative scenario (high consumption of breakfast cereals, highest concentration of 4-methylbenzophenone), a health concern could not be excluded.
The full Statement is available through: http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902360964.htm

For the current opinion the Panel after having examined the toxicological data on benzophenone considers that the liver hypertrophy seen in rat in the two-generation study is an adaptive response and not an adverse response. Therefore the Panel considered the EFSA approach to derive a LOAEL of 6 mg/kg b.w. per day based on this effect as conservative. Due to the limited amount of time available to EFSA for the release of its statement the Panel considered this conservative approach reasonable.

The Panel derives a TDI of 0.03 mg/kg b.w. per day for benzophenone based on the BMDL10 value of 3.1 mg/kg b.w. per day following BMD analyses on the non-neoplastic kidney effects in male rats. This value is proposed to be used as a basis for calculation of the Margin of Exposure (MoE) for 4-methylbenzophenone.
A factor of 100 for inter- and intraspecies differences in sensitivity and a factor of 2 for read-across from benzophenone to 4-methylbenzophenone are applied. Hence the estimated MoE should be greater than 200. The MoE is calculated by dividing the BMDL10 (3.1 mg/kg b.w. per day) by the estimated exposure in each case.

According to a highly conservative scenario as described in the EFSA statement (high consumption of breakfast cereals – highest concentration of 4-methylbenzophenone + assumptions for other foods) the highest dietary exposure to 4-methylbenzophenone is 15.2 µg/kg b.w. per day for children (EFSA, 2009).
The MoE for this exposure estimate as calculated against the BMDL10 value for benzophenone in this opinion is 204. (3100 divided by 15.2)
This MoE is not less that the minimum required MoE of 200.

The Panel concluded that short term consumption of contaminated breakfast cereals at current levels should not pose a risk to people. This conclusion is based on the limited exposure data available and read across from the toxicity of the similar substance benzophenone. If the use of 4-methylbenzophenone is to be continued, more data on occurrence of the substance in foods should be provided as well as appropriate toxicity data corresponding to the level of exposure for a full risk assessment.

CONCLUSIONS

A. Derivation of a TDI for benzophenone

Based on the negative *in vitro* and *in vivo* results the Panel concluded that benzophenone has no genotoxic potential.
Liver and kidney were identified as the primary target organs of benzophenone toxicity in rats and mice. The Panel considered neoplastic and non-neoplastic end-points in rats and in mice, obtained from chronic carcinogenicity (NTP, 2006) and reproductive toxicity assessments (Hoshino *et al.*, 2005).

Benzophenone caused liver hypertrophy in the rat at the lowest dose level (~6 mg/kg/day) in a two-generation study (Hoshino *et al.*, 2005), described as a slight hypertrophy of centrilobular hepatocytes without any increase in liver weight. However, in a chronic carcinogenicity study in rats benzophenone did not cause liver tumours, even at exposure levels yielding severe liver damage. Therefore the Panel considers that the liver hypertrophy seen in rat is an adaptive response and not an adverse response. In the B6C3F1 mouse, benzophenone causes liver adenomas at a dose of 40 mg/kg/day. The Panel considers the liver adenomas to be an adverse effect. However, this effect is a less sensitive endpoint than the kidney effects.

The Panel noted that benzophenone causes kidney adenoma in rat, associated with a spectrum of responses including hyperplasia and nephropathy at the lowest dose level of 15 mg/kg/day in a chronic carcinogenicity study (NTP, 2006). The Panel considered that the non-neoplastic kidney effects observed in the chronic assay were adverse. Benchmark dose (BMD) analyses were applied for the non-neoplastic kidney effects in male rats, and the lower 95% confidence limits of the bench mark dose for a 10% effect (BMDL10) were calculated to be 3.1 to 7.4 mg/kg b.w. per day (Appendix). The models used in the analysis were consistent, and passed statistical validation. The Panel decided that the BMDL10 value of 3.1 mg/kg b.w. per day was the most appropriate departure point for derivation of the TDI. By applying an uncertainty factor of 100, a TDI of 0.03 mg/kg b.w. per day is derived.

The Panel noted that the SCF had established for benzophenone and 4-hydroxybenzophenone a group TDI of 0.01 mg/kg body weight (SCF, 1992). The evaluation of the SCF was only based on a metabolism study and on a 90-day oral rat study on benzophenone.

4-Hydroxybenzophenone is one of the two important metabolites of benzophenone. However, the Panel considers that this fact alone, in the absence of supporting data, does not justify the inclusion of 4-hydroxybenzophenone in the same TDI with benzophenone.

B. Consequences of the new TDI for benzophenone on the risk assessment of 4-methylbenzophenone

For the purpose of providing an urgent advice to the risk managers, the EFSA in its statement considered the liver hypertrophy seen in the two-generation study (Hoshino *et al.*, 2005) with benzophenone as adverse effects. Thus, EFSA derived a LOAEL of 6 mg benzophenone/kg b.w. per day from this study.

For the current opinion the Panel after having examined the toxicological data on benzophenone considers that the liver hypertrophy seen in rat in the two-generation study is an adaptive response and not an adverse response. Therefore the Panel considered the EFSA

approach to derive a LOAEL of 6 mg/kg b.w. per day based on this effect as conservative. Due to the limited amount of time available to EFSA for the release of its statement the Panel considered this conservative approach reasonable.

The Panel derives a TDI of 0.03 mg/kg b.w. per day for benzophenone based on the BMDL10 value of 3.1 mg/kg b.w. per day following BMD analyses on the non-neoplastic kidney effects in male rats (NTP, 2006). This value is proposed to be used as a basis for calculation of the Margin of Exposure (MoE) for 4-methylbenzophenone.
A factor of 100 for inter- and intraspecies differences in sensitivity and a factor of 2 for read-across from benzophenone to 4-methylbenzophenone are applied. Hence the estimated MoE should be greater than 200. The MoE is calculated by dividing the BMDL10 (3.1 mg/kg b.w. per day) by the estimated exposure in each case.
According to a highly conservative scenario as described in the EFSA statement (high consumption of breakfast cereals – highest concentration of 4-methylbenzophenone + assumptions for other foods) the highest dietary exposure to 4-methylbenzophenone is 15.2 µg/kg b.w. per day for children (EFSA, 2009).
The MoE for this exposure estimate as calculated against the BMDL10 value for benzophenone in this opinion is 204. (3100 divided by 15.2)
This MoE is not less that the minimum required MoE of 200.

The Panel concluded that short term consumption of contaminated breakfast cereals at current levels should not pose a risk to people. This conclusion is based on the limited exposure data available and read across from the toxicity of the similar substance benzophenone. If the use of 4-methylbenzophenone is to be continued, more data on occurrence of the substance in foods should be provided as well as appropriate toxicity data corresponding to the level of exposure for a full risk assessment.

REFERENCES

Anderson, W. A. C. and Castle, L., 2003 'Benzophenone in cartonboard packaging materials and the factors that influence its migration into food', Food Additives & Contaminants: Part A, 20:6,607 - 618.

Buist, S.C., and Klaassen, C.D. (2004). Rat and mouse differences in gender-predominant expression of organic anion transporter (Oat1-3, SLc22a6-8) mRNA levels, Drug Metab. Dispos. 32, 620-625.

Burdock, G.A., Pence, D.H., and Ford, R.A. (1991). Safety evaluation of benzophenone. Food Chem.Toxicol. 29, 741-750.

CCRIS (Chemical Carcinogenesis Research Information System), 1991. On-line database of the National Library of Medicine's Toxicology Data Network (TOXNET®) maintained by the National Cancer Institute (NCI) at http://toxnet/nlm.nih.gov

Choi, J.O., Jitsunari, F., Asakawa, F., Park, H.J., Lee, D.S.,(2002). Migration of surrogate contaminants in paper and paperboard into water through polyethylene coating layer. Food Additives and Contaminants, 19 (12), pp. 1200-1206.

CoE (Council of Europe) Committee of Ministers, 2005. Resolution ResAP(2005)2 on packaging inks applied to the non-food contact surface of food packaging materials and articles intended to come into contact with foodstuffs (Adopted by the Committee of Ministers on 14 September 2005 at the 937th meeting of the Ministers' Deputies). Available at: http://www.coe.int/t/e/social_cohesion/soc-sp/public_health/food_contact/

EC (European Commission), 1992. First report of the Scientific Committee for Food on certain additives used in the manufacture of plastic materials intended to come into contact with foodstuffs (Opinion expressed on 3 May 1992).
Available at: http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_33.pdf

EC (European Commission), 2002. Commission Directive 2002/72/EC, relating to plastic materials and articles intended to come into contact with foodstuffs; http://europa.eu.int/comm/food/food/chemicalsafety/foodcontact/2002-72_en.pdf

EC (European Commission), Joint Research Centre, 2000. IUCLID dataset on benzophenone. http://ecb.jrc.ec.europa.eu/esis

EFSA (European Food Safety Authority), 2009. EFSA statement on the presence of 4-methylbenzophenone found in breakfast cereals, The EFSA Journal (2009) RN-243. http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902360964.htm

Feigenbaum, A., Dole, P., Aucejo, S., Dainelli, D., de la Cruz Garcia, C., Hankemeier, T., N'Gono, Y., Papaspyrides, C.D., Paseiro, P., Pastorelli, S., Pavlidou, S., Pennarun ,P.Y. Saillard, P., Vidal, L., Vitrac, O., Voulzatis, Y., 2005. Functional Barriers: properties and evaluation Food Additives and Contaminants, 2005, 22, 956-967.

Felisberti, M.-I., Giesse, R, and De Paoli, M.-A. 1985. Photoinitiation. Preparation of Low-Density Polyethylene. Composites by UV Irradiation. Polymer Bulletin 13, 163-169.

FSA (UK Food Standards Agency), 2000. Benzophenone from cartonboard, Food Surveillance. Information Sheet 6/00, October 2000.
Available at: http://www.food.gov.uk/science/surveillance/fsis2000/6benzo

FSA (UK Food Standards Agency), 2006. Benzophenone and 4-hydroxybenzophenone migration from food packaging into foodstuffs. Food Survey Information Sheet. Available at: http://www.food.gov.uk/science/surveillance/fsisbranch2006/fsis1806

Hoshino, N., Tani, E., Wako, Y., Takahashi, K., 2005. A two-generation reproductive toxicity study of benzophenone in rats. The journal of Toxicological Sciences, Vol. 30, Special Issue, 5-20.

Jeon, H-K., Sarma S. N., Kim Y-J., Ryu J-C., 2008. Toxicokinetics and metabolisms of benzophenone-type UV filters in rats, Toxicology, Volume 248, Issues 2-3, 89-95, 27 June 2008.

Jickells, S.M., Poulin, J., Mountfort, K.A., Fernàndez-Ocaña, M., 2005. Migration of contaminants by gas phase transfer from carton board and corrugated board box secondary packaging into foods. Food Additives and Contaminants, 22 (8), pp. 768-782.

Lei, J. and Liao, X., 2000. Surface graft copolymerization of acrylic acid onto LDPE film through corona discharge, European Polymer Journal 37, 771-779.

Mikamo, E., Harada, S., Nishikawa, J., Nishihara, T., 2003. Endocrine disruptors induce cytochrme P450 by affecting transcriptional regulation via pregnane X receptor. Toxicol Appl. Pharmacol. 193, 66-72.

Molina-Molina-J.-M., Escande, A., Pillon A., Gomez, E., Pakdel, F., Cavaillès, V., Olea, N., Ait-Aissa,A., Balaguer, P., 2008. Profiling of bezophenone derivatives using fish and human estrogen receptor-specific in vitro bioassays. Toxicol Appl. Pharmacol. 232, 384-395.

Nakagawa, Y., Suzuki T., Tayama S., 2000. Metabolism and toxicity of benzophenone in isolated rat hepatocytes and estrogenic activity of its metabolites in MCF-7 cells,Toxicology, Volume 156, Issue 1, 27-36, 7 December 2000.

Nakagawa, Y., and Tayama K., 2001. Estrogenic potency of benzophenone and its metabolites in juvenile female rats. Arch. Toxicol. 75, 74-79.

Nakagawa, Y., Tayama, K., 2002. Benzophenone-induced estrogenic potency in ovariectomized rats. Arch Toxicol. 2002 Dec;76(12):727-31. Epub 2002 Sep 25.

Nerín, C., Asensio, E., 2007. Migration of organic compounds from a multilayer plastic-paper material intended for food packaging. Analytical and Bioanalytical Chemistry, 389 (2), pp. 589-596.

NTP (National Toxicology Program), 2000. NTP technical report on the toxicity studies of benzophenone (CAS No. 119-61-9) administered in feed to F344/N rats and B6C3F1 mice. NTP Toxicity Report Series 61.

NTP (National Toxicology Program),,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. National Institute of Environmental Health Sciences, Research Triangle Park, NC.

NTP (National Toxicology Program), 2004a. 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. National Institute of Environmental Health Sciences, Research Triangle Park, NC.

NTP (National Toxicology Program), 2006. Toxicology and carcinogenesis studies of benzophenone (CAS No. 119-61-9) in F344/N rats and B6C3F1 mice. NTP TR 533. Available at: http://ntp.niehs.nih.gov/files/533_Web.pdf

Pastorelli, S., Sanches-Silva, A., Cruz, J.M., Simoneau, C., Losada, P.P. 2008. Study of the migration of benzophenone from printed paperboard packages to cakes through different plastic films. European Food Research and Technology, 227 (6), pp. 1585-1590.

Rhodes, M.C., Bucher J.R., Peckham J.C., Kissling G.E., Hejtmancik M.R., Chhabra R.S., 2007. Carcinogenesis studies of benzophenone in rats and mice. Food and Chemical Toxicology 45,843-851.

Schultz, T.W., Seward, J.R., Sinks, G.D., 2000. Estrogenicity of bezophenones evaluated with a recombinant yeast assay: Comparison of experimental and rules-based predicted activity. Environ. Toxicol. Chem. 19, 301-304

Song, Y.S., Begley, T., Paquette, K., Komolprasert, V., 2003. Effectiveness of polypropylene film as a barrier to migration from recycled paperboard packaging to fatty and high-moisture food. Food Additives and Contaminants, 20 (9), pp. 875-883.

Suzuki, T., Kitamura, S., Khota, R., Sugihara, K., Fujimoto, N., Ohata, S., 2005. Estrogenic and antiandrogenic activities of 17 benzophenone derivatives used as UV stabilzers and sunscreens. Toxicol. Appl. Pharmacol. 203, 9-17.

Takemoto, K., Yamazaki, H., Nakajima, M., and Yokoi, T., 2002. Genotoxic activation of benzophenone and its two metabolites by human cytochrome P450s in SOS/umu assay. Mutat. Res. 519, 199-204.

Triantafyllou, V.I., Akrida-Demertzi, K., Demertzis, P.G. 2007. A study on the migration of organic pollutants from recycled paperboard packaging materials to solid food matrices. Food Chemistry, 101 (4), pp. 1759-1768.

USEPA, (United States Environmental Protection Agency), 1984. Information Review; Benzophenone. Submitted by CRCS, Rockville, MD, in collaboration with Dynamac Corporation Environmental Control Division, Rockville, MD, to USEPA, TSCA Interagency Testing Committee.

APPENDIX

Benchmark dose analysis of Benzophenone

Kidney effects

1. The data

Male F344/N Rats

Doses 0, 15, 30, 60 mg/kg b.w. perday

Non-neoplastic kidney effects:

Standard/extended evaluations combined: 3/50, 11/50, 30/50, 40/50

Renal tubule, hyperplasia: 1/50, 5/50, 20/50, 23/50

Pelvis, transitional epithelium, hyperplasia 1/50, 11/50, 29/50, 34/50

Neoplastic kidney effects:

Standard and extended evaluations combined: 2/50, 2/50, 7/50, 8/50

2. Benchmark response (BMR) used in the analysis:

Extra risk = 0.10

3. Software used:

BMDS version 1.4.1c

4. Additional assumptions:

None

5. BMD results

Table 1. Non-neoplastic effects, kidney: standard /extended evaluations combined

Model used	No. of parameters	log-likelihood	p-value	accepted[1]	BMD_{10}	$BMDL_{10}$
Null model	1	-136.058	<.0001	no	-	-
Gamma	3	-97.4269	0.14	yes	8.48	4.01
Logistic	2	-99.7026	0.036	no	-	-
log-Logistic	3	-96.9273	0.29	yes	9.89	5.86
Probit	2	-99.6006	0.039	no	-	-
log-Probit	3	-96.9438	0.28	yes	10.4	6.35
Multi-stage (degree, n = 1)[2]	2	-98.5542	0.11	yes	4.37	**3.56**
Multi-stage (degree, n = 2)[2]	3	-97.8765				
Weibull	3	-97.5939	0.12	yes	7.51	3.91
Full model	4	-96.3645	-	-	-	-

[1] Model accepted if $p > 0.05$. The p-value results from a comparison of the log-likelihood for the model in question and that for the *Full model* under a likelihood ratio test. Rejecting the *Null model* (a horizontal line fitted to the data) suggests that there is a dose-response trend.

[2] In the BMDS software the user needs to specify the degree, n, of the polynomial term in the multi-stage model. The optimal degree has here been determined to be n = 1 under the likelihood ratio test. The BMD values for this case are presented in the Table. Use of a degree higher than n = 1 does not give a significantly better fit.

The range of BMDL values among the accepted models in Table 1 is 3.6 - 6.4 mg/kg b.w. per day.

Table 2. Non-neoplastic effects, kidney: renal tubule, hyperplasia

Model used	No. of parameters	log-likelihood	p-value	accepted[1]	BMD_{10}	$BMDL_{10}$
Null model	1	-111.355	<.0001	no	-	-
Gamma	3	-91.5751	0.033	no	-	-
Logistic	2	-95.0713	0.0031	no	-	-
log-Logistic	3	-91.3271	0.044	no	-	-
Probit	2	-94.4611	0.0058	no	-	-
log-Probit	3	-91.1522	0.055	yes	12.3	**5.50**
Multi-stage (degree, n = 1)[2]	2	-91.615	0.099	yes	9.47	7.40
Multi-stage (degree, n = 2)[2]	3	-91.615				
Weibull	3	-91.5957	0.032	no	-	-
Full model	4	-89.3039	-	-	-	-

[1] Model accepted if $p > 0.05$. The p-value results from a comparison of the log-likelihood for the model in question and that for the *Full model* under a likelihood ratio test. Rejecting the *Null model* (a horizontal line fitted to the data) suggests that there is a dose-response trend.

[2] In the BMDS software the user needs to specify the degree, n, of the polynomial term in the multi-stage model. The optimal degree has here been determined to be n = 1 under the likelihood ratio test. The BMD values for this case are presented in the Table. Use of a degree higher than n = 1 does not give a significantly better fit.

The range of BMDL values among the accepted models in Table 2 is 5.5 - 7.4 mg/kg b.w. per day.

Table 3. Non-neoplastic effects, kidney: pelvis, transitional epithelium, hyperplasia

Model used	No. of parameters	log-likelihood	p-value	accepted[1]	BMD_{10}	$BMDL_{10}$
Null model	1	-132.313	<.0001	no	-	-
Gamma	3	-98.3952	0.059	yes	5.34	4.13
Logistic	2	-104.291	0.00046	no	-	-
log-Logistic	3	-97.9195	0.11	yes	7.03	**3.07**
Probit	2	-103.777	0.00077	no	-	-
log-Probit	3	-97.9017	0.11	yes	7.66	3.39
Multi-stage (degree, n = 1)[2]	2	-98.4005	0.17	yes	5.05	4.13
Multi-stage (degree, n = 2)[2]	3	-98.4005				
Weibull	3	-98.4005	0.058	yes	5.06	4.13
Full model	4	-96.6054	-	-	-	-

[1] Model accepted if $p > 0.05$. The p-value results from a comparison of the log-likelihood for the model in question and that for the *Full model* under a likelihood ratio test. Rejecting the *Null model* (a horizontal line fitted to the data) suggests that there is a dose-response trend.

[2] In the BMDS software the user needs to specify the degree, n, of the polynomial term in the multi-stage model. The optimal degree has here been determined to be n = 1 under the likelihood ratio test. The BMD values for this case are presented in the Table. Use of a degree higher than n = 1 does not give a significantly better fit.

The range of BMDL values among the accepted models in Table 3 is 3.1 - 4.1 mg/kg b.w. per day.

Table 4. Neoplastic effects, kidney: standard and extended evaluations combined

Model used	No. of parameters	log-likelihood	p-value	accepted[1]	BMD_{10}	$BMDL_{10}$
Null model	1	-62.7912	0.057	yes[3]	-	-
Gamma	3	-59.7605	0.23	yes	42.4	24.6
Logistic	2	-59.9949	0.38	yes	49.0	36.2
log-Logistic	3	-59.7495	0.23	yes	42.0	23.5
Probit	2	-59.9364	0.40	yes	47.6	34.3
log-Probit	3	-59.6858	0.25	yes	41.2	**18.5**
Multi-stage (degree, n = 1)[2]	2	-59.7798	0.47	yes	41.8	24.5
Multi-stage (degree, n = 2)[2]	3	-59.7798				
Weibull	3	-59.7645	0.23	yes	42.4	24.6
Full model	4	-59.0261	-	-	-	-

[1] Model accepted if $p > 0.05$. The p-value results from a comparison of the log-likelihood for the model in question and that for the *Full model* under a likelihood ratio test. Rejecting the *Null model* (a horizontal line fitted to the data) suggests that there is a dose-response trend.

[2] In the BMDS software the user needs to specify the degree, n, of the polynomial term in the multi-stage model. The optimal degree has here been determined to be n = 1 under the likelihood ratio test. The BMD values for this case are presented in the Table. Use of a degree higher than n = 1 does not give a significantly better fit.

[3] Strictly speaking, the p-value for the *Null model* does not support a dose-response trend. Consideration of the BMD and BMDL values in Table 4 would require the support of a dose-response trend by other means.

The range of BMDL values among the accepted models in Table 4 is 19 - 36 mg/kg b.w. per day.

6. Figures

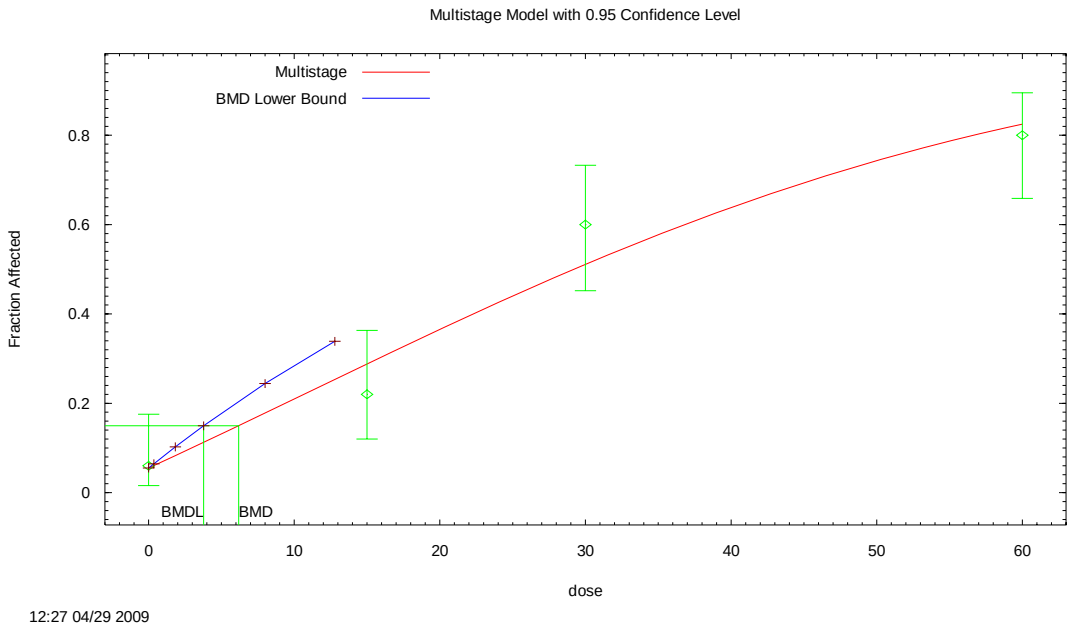


Figure 1. The multi-stage model (n = 1) fitted to "standard/extended evaluations combined" (non-neoplastic effects, kidney) observed in male F344/N rats. The BMDL is 3.6 mg/kg b.w. per day, which was the lowest BMDL obtained for this endpoint.

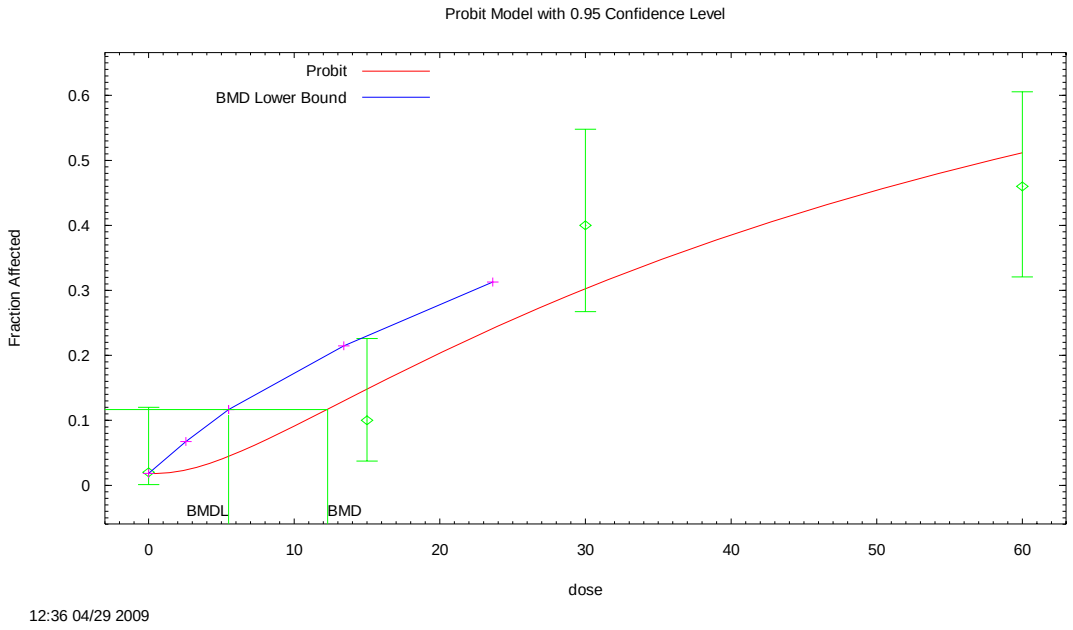


Figure 2. The log-probit model fitted to "renal tubule, hyperplasia" (non-neoplastic effects, kidney) observed in male F344/N rats. The BMDL is 5.5 mg/kg b.w. per day, which was the lowest BMDL obtained for this endpoint.

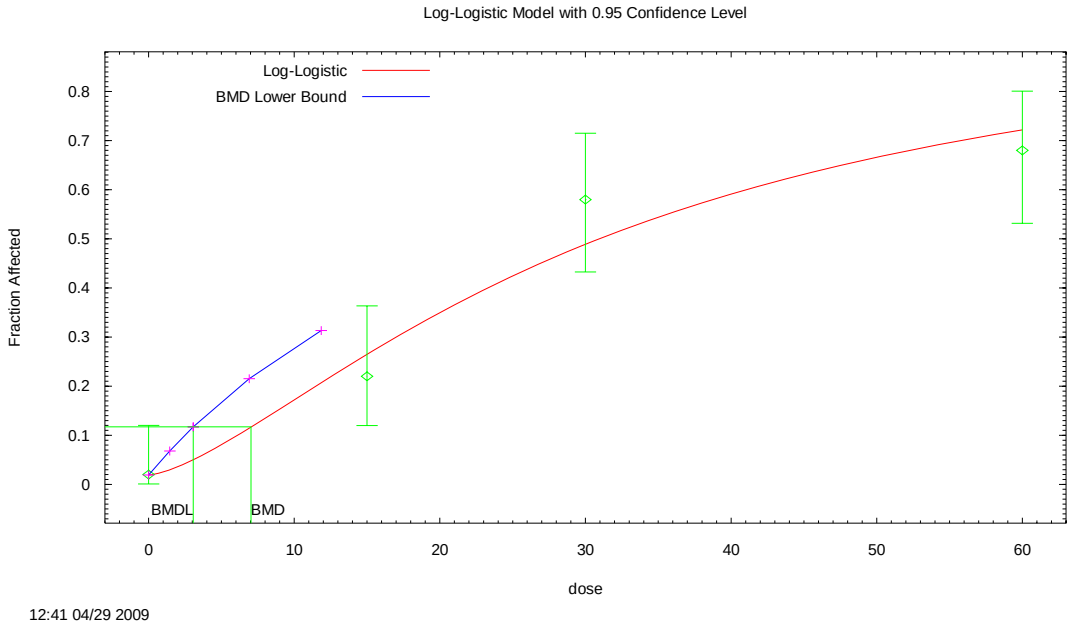


Figure 3. The log-logistic model fitted to "pelvis, transitional epithelium, hyperplasia" (non-neoplastic effects, kidney) observed in male F344/N rats. The BMDL is 3.1 mg/kg b.w. per day, which was the lowest BMDL obtained for this endpoint.

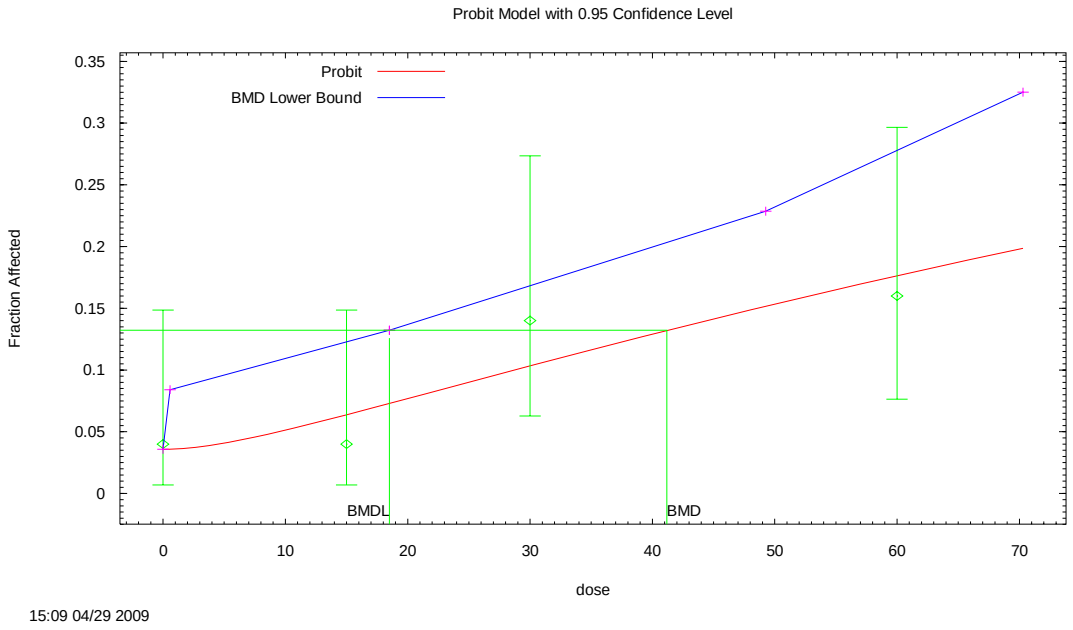


Figure 4. The log-probit model fitted to "standard and extended evaluations combined" (neoplastic effects, kidney) observed in male F344/N rats. The BMDL is 19 mg/kg b.w. per day, which was the lowest BMDL obtained for this endpoint. A dose-response trend was not statistically supported. Consideration of the BMD and BMDL values for this endpoint would require the support of a dose-response trend by other means.

7. Conclusion

For each endpoint, the differences between the BMD values, and BMDL values, among the accepted models are not very large. Thus, the result for the respective endpoint is quite stable even though there is slight extrapolation involved for some of the endpoints; where the BMR of 0.10 is lower than the observed response at the lowest dose.

The most conservative BMDL is 3.1 mg/kg b.w. per day.