

ROBUST STUDY SUMMARIES:
Critical Studies Cited in the 1,1'-Biphenyl (Biphenyl) Targeted Assessment Profile
for Human Health but Not Referenced in IPCS (1999: Biphenyl) or
WHO (2006: Safety evaluation of certain food additives)

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1.0 Genotoxicity

CHEMICAL:

Name: 1,1'-Biphenyl (CAS RN 92-52-4)

Purity: 99.5% (biphenyl obtained from Merck; Darmstadt, Germany)

METHOD:

Method/Guideline: IPCS Guidelines (Albertini et al. 2000)

Type of study: Chromosomal aberrations (CAs), Sister chromatid exchanges (SCEs) and Micronucleus (MN) test.

GLP: Not stated.

Year: 2008

In vitro study: Human lymphocytes (cell culture)

Analyses conducted: Whole blood from four healthy volunteers (two male and two female) was used to obtain lymphocytes. These human peripheral lymphocytes were treated with four concentrations of biphenyl [10, 30, 50, and 70 µg/mL – dissolved in dimethyl sulfoxide (DMSO)] for 24- and 48-h. A negative control (untreated cells), a solvent control (8 µL/mL DMSO) and a positive control (0.2 µg/mL of mitomycin C) were also used. Chromosomal aberrations were determined by calculating the percentage of metaphases from each treatment period that showed structural and/or numerical alterations (according to IPCS classification). The scoring of SCEs was also conducted according to the IPCS guidelines. The results were used to determine the mean number of SCEs (SCE/cell), and a replication index (RI) and mitotic index (MI) was determined. The micronucleus analysis was conducted to determine the nuclear division index (NDI) to estimate the cytotoxicity of biphenyl in human lymphocytes.

Statistical analysis: A student t-test was used to determine the statistical significance of all parameters and correlation or regression analyses were conducted to estimate the dose-response relationships.

RESULTS:

Exposure to biphenyl significantly increased the frequency of SCEs, CAs, and MN in a dose-dependent manner when compared with both untreated and solvent (DMSO) control. The effects of biphenyl were generally seen at concentrations higher than 10 µg/mL. Biphenyl induced the structural CAs instead of numerical CAs following 24 hr of 50 or 70 µg/mL of exposure to biphenyl, but not after 48 hr of exposure. Mean sister chromatid exchange was increased 24 hr after 50 or 70 µg/mL or 48 hr after 30, 50 or 70 µg/mL dose. Induction of micronuclei was seen 24 hr after exposure to 30, 50 or 70 µg/mL of biphenyl or after 48 hr following treatment with 50 or 70 µg/mL dose. Biphenyl also caused a cytotoxic effect by decreasing the replication index (RI) at the highest two concentrations for 48 h and nuclear division index (NDI) at the highest two concentrations for the 24- and 48-h treatment periods. However, biphenyl treatment did not affect the mitotic index (MI).

CONCLUSIONS:

According to the authors “biphenyl has a genotoxic effect and most probably might have a potential risk for humans”.

RELIABILITY:

(2) Valid with restrictions (Published article – not all study details presented).

GENERAL REMARKS:

This (*in vitro*) study suggested clastogenic effects of biphenyl in human lymphocytes. Available data showed equivocal results of the effects of biphenyl in other *in vitro* genotoxicity studies.

REFERENCES:

Rencuzogullari E, Parlak S, Ila HB. 2008. The effects of food protector biphenyl on sister chromatid exchange, chromosome aberrations, and micronucleus in human lymphocytes. *Drug Chem. Toxicol.* 31:263-274.

Albertini, R. J., Anderson, D., Douglas, O. R., Hagmar, L., Hemminki, K., Mono, F., Natarajan, A. T., Norppa, H., Shuker, D. E. G., Tice, R., Waters, M. D., Aitia, A. 2000. IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. *Mutat. Res.* 463:111—172.

CHEMICAL:

Name: 1,1'-Biphenyl (CAS RN 92-52-4)

Purity: 99.5% (biphenyl obtained from Tokyo Kasei Kogyo Industry Ltd, Tokyo, Japan)

METHOD:

Method/Guideline: Comet assay (modified). Followed the guidelines by Tyce et al (2000).

Type of study: DNA damage

GLP: Not stated.

Year: 2002.

Species and strain: Male ddY mice (Japan SLC Co., Shizuoka, Japan).

Dose/Concentrations: 10, 100, 1000 or 2000 mg/kg bw of biphenyl administered orally.

Analyses conducted: Mice were orally exposed to biphenyl (10, 100, 1000, or 2000 mg/kg bw).

Animals were sacrificed 3 or 24 hr after the exposure. Necropsies were performed glandular stomach, colon, liver, kidney, urinary bladder, lung, brain and bone marrow were removed. All tissues were prepared for comet assay and if results were positive, slides were prepared for histopathological examination.

Statistical analysis: Differences between the averages of four treated animals and the untreated control animals were compared with Dunnett test following one-way ANOVA. A p value < 0.05 was considered statistically significant.

RESULTS:

No signs of toxicity or mortality were seen in any treatment group. Similarly, necropsy or histopathological examination of tissue did not reveal any treatment related effects.

Biphenyl induced DNA damage in a dose related manner in the colon and the lowest dose that induced the DNA damage was 100 mg/kg. Exposure to biphenyl at 1000 mg/kg bw induced DNA damage in all the organs the organs examined in this study.

CONCLUSIONS:

The comet assay was positive (DNA damage) in the organs examined following 24 hr of single high-dose exposure to biphenyl. Despite the positive comet assay, the tumor incidence was rare in colon, glandular stomach and urinary bladder. The authors proposed that the comet assay detected the DNA damage shortly after the high-dose exposure to biphenyl, while carcinogenicity is detected after long-term low-dose exposure

RELIABILITY:

(2) Valid with restrictions (Published article – not all study details presented).

GENERAL REMARKS:

This showed that a single short-term high-dose exposure to biphenyl may cause DNA damage in various organs in mice. However, evidence of *in vivo* genotoxicity does not always reflect carcinogenicity.

REFERENCE:

Sasaki YF, Kawaguchi S, Kamaya A, Ohshita M, Kabasawa K, Iwama K, Taniguchi K, Tsuda S. 2002. The comet assay with 8 mouse organs: results with 39 currently food additives. *Mut. Res.* 519:103-119.

Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamac Y, Rojas E, Ryu JC, Sasaki YF. 2000. Single-cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. *Environ. Mol. Mutagen.* 35:206-221.

2.0 Mode of Action (Carcinogenicity)

CHEMICAL:

Name: 1,1'-Biphenyl (CAS RN 92-52-4)

Purity: > 98% (biphenyl obtained from Wako Pure Chemical Industries, Ltd. Osaka, Japan)

METHOD:

Method/Guideline: Carcinogenicity study conducted by following OECD TG 453 (OECD 1981).

Type of study: Combined chronic toxicity/carcinogenicity.

GLP: Yes

Year: Study ended on or before 1996.

Species and strain: Male and female Crj:BDF₁ mice (SPF), Charles River, Japan Inc. Kanagawa.

Dose/Concentrations: Biphenyl added to diet at 0, 667, 2000, or 6000 ppm (equal to 0, 97, 291, or 1050 mg/kg-bw per day in males and 0, 134, 414. or 1420 mg/kg-bw per day in females, respectively) for 2 years.

Analyses conducted: Mice were monitored for survival, changes in body weight, food consumption and signs of toxicity during the study. At study termination, hematological and blood biochemical parameters were measured and pre-neoplastic, neoplastic or histopathological lesions were examined in liver and kidney of male or female mice in all dose groups.

Statistical analysis: Changes in body weight, food consumption, organ weight, hematological and blood biochemical changes were analyzed by Dunnett's test. Incidences of non-neoplastic lesions were analyzed by Fisher's exact test and neoplastic lesions were analysed by applying Peto's trend test and Fisher's exact test. $P < 0.05$ was considered to be statistically significant.

RESULTS:

Food consumption: No treatment related changes.

Mortality: No treatment related changes.

Clinical appearance: No treatment related changes.

Body weights: Body weights (body-weight gain) were significantly decreased at 6000 ppm in both sexes throughout the study.

Organ weights: Relative liver weights in females were significantly increased by 1.3-, 1.4- and 1.6-fold at 667, 2000 and 6000 ppm, respectively.

Haematology and Serum biochemistry: No treatment related changes in haematology. Significant increases in serum enzymes (GOT and GPT in females; ALP in both sexes at 6000 ppm), blood urea nitrogen (males; increased in females at 6000 ppm) and calcium (females; sodium increased in both sexes at 6000 ppm) were noted at 2000 ppm and above in both sexes. "...females bearing the malignant liver tumor had extremely high serum levels of GOT, GPT and LDH."

Gross pathology: Dose-related increase in liver nodules in females. Number of females with liver nodules in which a proliferative lesion was histopathologically observed was 5/50, 5/50, 16/50, 19/49 at 0, 667, 2000 or 6000 ppm biphenyl, respectively.

Pathology: Female mice had significant increases in the incidence of hepatocellular adenomas at 2000 and 6000 ppm (incidences of 2/50, 3/50, 12/50 and 10/49 at 0, 667, 2000 or 6000 ppm biphenyl, respectively) and in hepatocellular carcinomas at 2000 ppm (incidences of

1/50, 5/50, 7/50 and 5/49 at 0, 667, 2000 or 6000 ppm biphenyl, respectively). There were no increases in tumour incidences in male mice.

Non-neoplastic effects in the liver was an increased incidence of basophilic cell foci in females at 2000 and 6000 ppm (incidences of 1/50, 1/50, 16/50, 14/50, respectively). Non-neoplastic effects observed in the kidneys included a significant increase in necrotic desquamation of urothelium in the renal pelvis in both sexes at 6000 ppm (incidences of 0/50, 0/49, 0/50 and 10/50 in males and 4/50, 0/50, 0/50 and 15/49 in females, respectively) and mineralization in the inner stripe of the outer medulla in females at 2000 ppm and above (incidences of 3/50, 5/50, 12/50 and 26/49, respectively).

CONCLUSIONS:

Long-term dietary exposure to biphenyl caused dose-related increase in the benign and malignant hepatocellular tumors and the pre-neoplastic liver lesions in the female mice together with non-neoplastic kidney lesions in both male and female mice.

RELIABILITY:

(2) Valid with restrictions (Published article – not all study details presented).

MODE OF ACTION:

The authors (Umeda et al. 2005) acknowledged the species and sex differences in the development of biphenyl-induced carcinogenicity in male and female rats or mice. Possible mode(s) of action were discussed such as:

1. In mice, biphenyl-induced hepatocarcinogenicity was attributed to peroxisome proliferation. “One of the causative factors for the biphenyl-induced hepatocarcinogenicity in the female mice was explored by the electron-microscopic study of 13-week oral administration of biphenyl-containing diets to male and female BDF₁ mice [Umeda et al. 2004]. The liver of the females fed diet containing biphenyl was histopathologically characterized by clearly enlarged hepatocytes filled with eosinophilic fine granules which were found to correspond to the increased number of peroxisomes by electron microscopy. The eosinophilic fine granules were not found in the liver of the male mice fed diets containing biphenyl. The present electron-microscopic finding of the biphenyl-induced peroxisome proliferation of the females was consistent with the result of Sunouchi *et al.* [1999] that oral administration of biphenyl to female BDF₁ mice increased the peroxisomal fatty acid β -oxidation activity, while the β -oxidation activity was not increased in the male mice...Long-term administration of many peroxisome proliferators to rodents has been known to cause the liver tumors. Therefore, it is thought that the peroxisome proliferation in the liver was involved in the biphenyl-induced hepatocarcinogenicity in female mice.”
2. In mice, biphenyl-induced hepatocarcinogenicity may be associated with possible DNA damage by formation of reactive biphenyl metabolites, i.e., 2-hydroxyl-biphenyl (2-HBP), 2,5-dihydroxy-biphenyl (2,5-DHBP) or 2-Phenyl-1,4-benzoquinone (2-PBQ). “Although mammalian cell clastogenicity of biphenyl was negative without S9 activation, it was positive in Chinese hamster V79 cell with the liver homogenate from rats and in Chinese hamster CHL/IU cell with S9 activation of mice. Biphenyl was positive in a micronucleus test with Chinese hamster cells in the S9 activation, suggesting positive clastogenicity by biphenyl metabolites. 2-Phenyl-1,4-benzoquinone (2-PBQ), an active metabolite of 2-hydroxyl-biphenyl (2-HBP), was reported to cross-link to DNA and to damage DNA in the epithelial cells of the rat urinary bladder and in the mouse liver, bladder and other organs. Therefore, it is inferred on the basis of the above literature that the genotoxic mode of action operates in the biphenyl-induced hepatocarcinogenicity, mediating through the possible DNA damage by active biphenyl metabolites.”

3. The differences in carcinogenic results between rats and mice is probably related to differences in the metabolic pathway between the two species.
- “Biphenyl was reported to be hydrolyzed in the liver at the first phase, conjugated with sulfate or glucuronide at the second phase, and to be finally excreted into urine. At the first phase, biphenyl was biotransformed to 4-hydroxyl-biphenyl (4-HBP) as major metabolic pathway in rats and mice, whereas the quantity of 2-HBP metabolized from biphenyl as the minor pathway was smaller in rats than in mice. It is, therefore, suggested that the lack of of the biphenyl-induced hepatocarcinogenicity in rats was attributable to the major metabolic pathway of biphenyl to 4-HBP but not to 2-HBP in mature rats, whereas 2-HBP was further metabolized to 2,5-DHBP, a possible peroxisome proliferator, and 2-PBQ, a genotoxicant [in mice]. It was reported that the second phase metabolic pathway of 4-HBP to its sulphate conjugate was causally related to the calculus formation in the bladder of the male rats fed a diet containing biphenyl, leading to the development of bladder tumors. This is in sharp contrast to the present finding of the lack of calculus formation and bladder tumor in the mice fed diets containing biphenyl. It is inferred, therefore, that the lack of the bladder tumor in the mice fed diets containing biphenyl is causally related to the lack of bladder calculi. However, the mouse biphenyl metabolism relating to the lack of calculus formation in the bladder remains to be solved for elucidation of the species differences in the bladder tumor.”

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- Sunouchi M, Miyajima A, Ozawa S, Ohno Y. 1999. Effects of diphenyl on hepatic peroxysomal enzyme and drug-metabolizing enzymes activities in BDF₁ mice. *J. Toxicol. Sci.* 24: 333
- Umeda Y, Shigetoshi A, Arito H, Kasuke N, Matsushima T. 2004. Induction of peroxisome proliferation in the liver of biphenyl-fed female mice. *J. Occup. Health.* 46: 486-488.
- Umeda Y, Shigetoshi A, Yamazaki K, Ohnishi M, Arito H, Kasuke, N, Yamamoto S, Matsushima T. 2005. Carcinogenicity of biphenyl in mice by two years feeding. *J. Vet. Med. Sci.* 67(4): 417-24.
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