Influence of Five Methylsulphonyl PCB Congeners on Frequency of Micronucleated Cells in Cultured Human Lymphocytes by Cytokinesis Block Method

NAGAYAMA, Junya
Laboratory of Environmental Health Sciences, School of Health Sciences, Kyushu University

NAGAYAMA, Mayumi
Laboratory of Environmental Health Sciences, School of Health Sciences, Kyushu University

HARAGUCHI, Koichi
Daiichi College of Pharmaceutical Sciences

KUROKI, Hiroaki
Daiichi College of Pharmaceutical Sciences

他

https://doi.org/10.15017/7323618

出版情報：福岡醫學雜誌. 86 (5), pp.190-196, 1995-05-25. 福岡医学会
バージョン：
権利関係：

Influence of Five Methylsulphonyl PCB Congeners on Frequency of Micronucleated Cells in Cultured Human Lymphocytes by Cytokinesis Block Method

Junya NAGAYAMA and Mayumi NAGAYAMA
Laboratory of Environmental Health Sciences, School of Health Sciences, Kyushu University 90, Fukuoka 812-82
Koichi HARAGUCHI, Hiroaki KUROKI and Yoshito MASUDA
Daiichi College of Pharmaceutical Sciences, Fukuoka 815

Abstract The lungs and blood of Yusho patients and healthy Japanese people have already been contaminated with methylsulphonyl polychlorinated biphenyls (MSF-PCBs) at relatively high concentration. Therefore, we should give due attention to their biological and toxicological effects to man. In this study, in order to mainly evaluate non-S-dependent genotoxicity of five MSF-PCB congeners, namely, 3-MSF-4, 5, 3', 4'-tetrachlorobiphenyl (TCB), 3-MSF-4, 5, 2', 3'-TCB, 3-MSF-2, 5, 2', 4', 5'-pentachlorobiphenyl (PenCB), 4-MSF-2, 5, 2', 3', 4'-PenCB and 4-MSF-2, 5, 2', 3', 5', 6'-hexachlorobiphenyl (HCB). We have examined their effects on the induction of micronucleated cells, which has been frequently used to estimate the dose of ionizing radiation and truly radiomimetic, non-S-dependent, clastogens, in cultured human lymphocytes in the absence or presence of 2, 3, 4, 7, 8-pentachlorodibenzofuran (PenCDF), 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) or 3, 4, 5, 3', 4'-pentachlorobiphenly (Co-PenCB). The following results were obtained. 1) 4×10^{-5} M7, 8-benzoflavone (ANF) significantly enhanced the frequency of micronucleated cells and all of the five MSF-PCB congeners failed to induce the formation of micronucleated cells at doses of 5.2 to 9.6 ppm, which were about 35,000 times higher than the concentrations in the lungs and adipose tissue of healthy Japanese people. 2) In the simultaneous treatment of one of the five MSF-PCB congeners and PenCDF (3.9 ppb), TCDD (1.5 ppb) or Co-PenCB (8.8 ppb), the combination of 3-MSF-2, 5, 2', 4', 5'-PenCB (5.2 ppm) and PenCDF only significantly enhanced the formation of micronucleated cells and the frequency rate was two times greater than that of the control culture, which was treated with PenCDF alone.

Based on the results of this study, the five MSF-PCB congeners examined are considered not to be or very weak non-S-dependent genotoxic chemicals. We, however, have not studied yet whether some MSF-PCBs are S-dependent genotoxic compounds or not. Therefore, their effects on the induction of sister chromatid exchanges (SCEs), which has proved to be the most sensitive mammalian endpoint for determining exposure to S-dependent DNA insults, in cultured human lymphocytes are now under investigation in our laboratory.

Introduction

Methylsulphonyl polychlorinated biphenyls (MSF - PCBs), which are one of the major metabolites of polychlorinated biphenyls (PCBs), have been determined in several tissues of patients with Yusho, polychlorinated dibenzofurans (PCDFs) poisoning that occurred in western Japan in 1968, and of healthy Japanese people[6)7)8)9)10)]. According to their analysis, the concentrations of MSF-PCBs were much lower than those of PCBs in the liver and adipose tissues of both Yusho patients and healthy people. However, in the lungs and blood their

concentrations were comparable to or higher than those of PCBs. Thus, several tissues of Japanese people have already been contaminated with MSF-PCBs at relatively high concentration. Therefore, we should give due attention to their biological and toxicological action.

We have already investigated the effects of some MSF-PCB congeners on aryl hydrocarbon hydroxylase (AHH) activity in cultured human lymphoblastoid cells and in hepatic microsomes prepared from both aryl hydrocarbon (Ah) responsive and nonresponsive strains of mice[15)16)17)18)19)25)]. Our studies mentioned above indicated that some congeners of MSF-PCBs decreased and/or enhanced the enzyme activity in both cultured human lymphoblastoid cells and murine hepatic microsomes. Therefore, some MSF-PCB congeners have been expected to elicit biological or toxicological effect in man.

Micronuclei, which have been thought to reflect chromosomal or genetic damage from carcinogenic insults such as smoking and irradiation, enclose acentric chromosome fragments or whole chromosomes that have not been incorporated in the main nuclei at cell division, and enumeration of micronuclei or micronucleated cells in mitogen-stimulated lymphocytes provides a simpler and statistically more precise method than karyotypic analysis for quantitation of chromosomal damage. A cytokinesis-block method, using cytochalasin B (CYB), an inhibitor of actin assembly[1)], was firstly reported in 1985[5)], and the prevention of cytoplasmic division after nuclear division by CYB, resulting in binucleated cells, facilitates the scoring of micronuclei or micronucleated cells and increases the precision of the assay.

In this study, we investigated the induction of micronucleated cells in mitogen-stimulated lymphocytes by five MSF-PCB congeners, which were major ones among MSF-PCBs determined in the tissues of Yusho patients and healthy Japanese people[8)], in order to elucidate their genotoxicity in terms of inducer of micronucleated cells.

Materials and Methods

1. Chemicals

Five MSF-PCB congeners, 3-MSF-4, 5, 3', 4'-tetrachlorobiphenyl (TCB), 3-MSF-4, 5, 2', 3'-TCB, 3-MSF-2, 5, 2', 4', 5'-pentachlorobiphenyl (PenCB), 4-MSF-2, 5, 2', 3', 4'-PenCB and 4-MSF-2, 5, 2', 3', 5', 6'-hexachlorobiphenyl (HCB), 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 2, 3, 4, 7, 8-pentachlorodibenzofuran (PenCDF) were synthesized by Dr. Masuda and his colleagues. 3, 4, 5, 3', 4'-Pentachlorobiphenyl (Co-PenCB) was purchased from Cambridge Isotope Laboratories, Woburn, USA. Their purities were over 99% by gas chromatography. The sources of chemicals used in this work were as follows: 7, 8-benzoflavone (ANF) from Wako, Osaka, cytochalasin B (CYB) from Sigma, St. Louis, phytohemagglutinin M (PHA) from Difco, Detroit, penicillin, streptomycin and RPMI 1640 from Gibco, New York. All other chemicals and reagents were of the highest quality commercially available.

2. Chemical treatment and lymphocyte culture

Final concentrations of test chemicals in whole-blood cultures were as follows: 3-MSF-4, 5, 3', 4'-TCB; 6.8 ppm, 3-MSF-4, 5, 2', 3'-TCB; 7.1 ppm, 3-MSF-2, 5, 2', 4', 5'-PenCB; 5.2 ppm, 4-MSF-2, 5, 2', 3', 4'-PenCB; 5.8 ppm, 4-MSF-2, 5, 2', 3', 5', 6'-HCB; 9.6 ppm, TCDD; 1.5 ppb, PenCDF; 3.9 ppb, Co-PenCB; 8.8 ppb and ANF; 4×10^{-5}M (11 ppm).

Lymphocyte cultures (5.0 ml) were initiated from whole blood (0.3 ml) aliquated into culture tubes containing the test chemical(s) as described later and RPMI 1640 medium (4.5 ml) supplemented with 15% fetal calf serum, 100 units/ml penicillin and 100μg/ml streptomycin.

Firstly, these whole-blood cultures were incubated with either acetone (solvent) alone, ANF or one of the five MSF-PCBs. Secondly, the whole-blood cultures were treated with one

of the five MSF-PCB congeners in the presence of either TCDD, PenCDF or Co-PenCB. These test chemicals were added at the beginning of the culture time and left until harvesting.

PHA (0.15 ml and a final concentration: 3%) was added at culture initiation. The whole-blood cultures were then incubated at 37°C in 5% CO_2 and 100% humidity. At 71 hrs after the commencement of the culture, 7μM CYB was added and the incubation was further continued for additional 26 hrs.

3. Slide preparation and scoring

At the end of incubation, the whole-blood cultures were centrifuged (1,000 rpm×5 min), resuspended in 0.1M KCl hypotonic solution for 15 min at 37°C and centrifuged. Then, cells were fixed 3 times in fresh fixative (methanol: acetic acid, 3 : 1) at room temperature. Samples for microscopic observation were obtained by carefully dropping cell suspension from a Pasteur pipette onto wet clean slides. Slides were dried in vapor of water and stained with 3% Giemsa (Merck) in distilled water.

Slides were coded and scored blind under magnification of 1,000 fold. A total of 1,500 binucleated lymphocytes with preserved cytoplasm were scored for each experimental group. Criteria for evaluation of micronucleated cells were those suggested by Countryman and Heddle[3].

4. Statistical analysis

The results are expressed as the average number±S. E. of micronucleated cells from 3 observations of 500 binucleated cells each on different slides from the same group. Data were statistically analyzed by Student's t-test.

Results

1. Frequency of micronucleated cells induced by each of the five MSF-PCB congeners in the absence or presence of PenCDF.

The experimental results are shown in Table 1. The frequency of micronucleated cells in the control culture was 2.7±0.4 per 500 binucleated lymphocytes, and the treatment of PenCDF (3.9 ppb) did not enhance the micronuclei formation. ANF(4×10^{-5}M), however, significantly induced the micronucleated cells and the frquency rate was 4.3±0.4 per 500 binucleated lymphocytes.

In the five MSF-PCB congeners, significant increase of the micronucleated cells was not observed after their single treatment in the absence of PenCDF, and the range of micronucleated cell rates was 2.7 to 4.0 per 500 binucleated lymphocytes. However, in the presence of PenCDF, 3-MSF-2, 5, 2', 4', 5'-PenCB only significantly enhanced the frequency of micronucleated cells and the rate was 4.0±0.7 per 500 binucleated lymphocytes even at the lowest

Table 1 Statistical analysis of the effect of the five MSF-PCB congeners on micronucleated cell rates in the absence or presence of PenCDF

MSF-PCB congener and ANF	Dose(ppm)	Micronucleated cells (per 500 binucleated lymphocytes)	
		PenCDF(-)	PenCDF(3.9 ppb)
Control	−	2.7±0.4	2.0±0.4
3-MSF-4,5,3',4'-TCB	6.8	2.7±0.4	2.7±0.4
3-MSF-4,5,2',3'-TCB	7.1	3.0±0.7	3.0±0.7
3-MSF-2,5,2',4',5'-PenCB	5.2	3.7±0.4	4.0±0.7*
4-MSF-2,5,2',3',4'-PenCB	5.8	3.3±0.4	2.7±0.4
4-MSF-2,5,2',3',5',6'-HCB	9.6	4.0±0.7	3.3±0.4
ANF	11	4.3±0.4*	−

*: Significantly different from respective control figures, $p<0.05$

dose (5.2 ppm) among the MSF-PCB congeners. The rest of them did not show any significant effect on the induction of the micronucleated cells.

2. Frequency of micronucleated cells induced by each of the five MSF-PCB congeners in the presence of TCDD or Co-PenCB.

The experimental results are indicated in Table 2. Respective micronucleated cell rates after the treatment of TCDD (1.5 ppb) and Co-PenCB (8.8 ppb) were 3.7±0.4 and 2.3±0.8 per 500 binucleated lymphocytes and these rates were not significantly different from that in control culture, which was 2.7±0.4, as shown in Table 1.

Simultaneous treatment of TCDD and one of the five MSF-PCB congeners did not show any significant effect on the induction of the micronucleated cells and the range of the frequency rates was 3.3 to 4.7 per 500 binucleated lymphocytes. In the presence of TCDD, 3-MSF-2, 5, 2', 4', 5'-PenCB showed the highest frequency rate per 1 ppm dose in the five MSF-PCB congeners.

Simultaneous treatment of Co-PenCB and one of the five MSF-PCB congeners did not significantly enhance the formation of micronucleated cells and the range of the frequency rates was 3.3 to 3.7 per 500 binucleated lymphocytes. As in the case of TCDD, the highest rate per 1 ppm dose was observed after the simultaneous treatment of 3-MSF-2, 5, 2', 4', 5'-PenCB and Co-PenCB.

Discussion

We have already reported that ANF and 3-MSF-4, 5, 3', 4'-TCB, one of the MSF-PCB congeners examined in this study, inhibit or enhance aryl hydrocarbon hydroxylase (AHH) activity in mice and human lymphoblastoid cells, probably depending on the quantity and quality of cytochrome P-450 (P-450) enzymes, which catalyze benzo(a)pyrene (BP), a substrate of the AHH assay, metabolism[15)16)17)19)25)]. We have also investigated the effect of eleven MSF-PCB congeners on AHH activity of cultured human lymphoblastoid cells treated with TCDD and demonstrated that the TCDD-induced enzyme activity was mostly inhibited with them by 10 to 80%, depending on congeners[18)]. Three of the eleven MSF-PCBs were 3-MSF-4, 5, 3', 4'-TCB, 3-MSF-4, 5, 2', 3'-TCB and 4-MSF-2, 5, 2', 3', 4'-PenCB, which were used in this study, and respective rates of the enzyme inhibition were about 80%, 40% and 20% at the dose of 1.5μg/ml, in other words, 1.5 ppm which was about 4 to 5 times lower concentration than those used in this study, namely 5.8 to 7.1 ppm. In that experiment, ANF showed the highest AHH inhibition at the dose of 1.4μg/ml (1.4 ppm), which was about 8 times lower concentration than that (4×10^{-5}M) used

Table 2 Statistical analysis of the effect of the five MSF-PCB congeners on micronucleated cell rates in the simultaneous treatment of TCDD or Co-PenCB

MSF-PCB congener	Dose(ppm)	Micronucleated cells (per 500 binucleated lymphocytes)	
		TCDD (1.5 ppb)	Co-PenCB (8.8 ppb)
Control	—	3.7±0.4	2.3±0.8
3-MSF-4,5,3',4'-TCB	6.8	3.3±0.4	3.3±0.4
3-MSF-4,5,2',3'-TCB	7.1	3.7±0.4	3.7±0.4
3-MSF-2,5,2',4',5'-PenCB	5.2	4.7±1.1	3.3±0.8
4-MSF-2,5,2',3',4'-PenCB	5.8	3.7±0.4	3.3±0.4
4-MSF-2,5,2',3',5',6'-HCB	9.6	4.7±0.8	3.7±0.8

in this study, that is, about 11μg/ml (11 ppm) and the inhibition rate was about 90%. Like this, some MSF-PCB congeners such as 3-MSF-4, 5, 3', 4'-TCB and 3-MSF-4, 5, 2', 3'-TCB have been expected to demonstrate the same biological action as ANF.

At doses of 4×10^{-5}M to 8×10^{-5}M, ANF has been shown to elicit some clastogenic or co-clastogenic potency[2)12)13)14)20)21)22)23)24)], so we anticipated that some MSF-PCB congeners including those mentioned above also could evoke similar clastogenic or co-clastogenic effect to ANF and this study was carried out. As the results of this study, 4×10^{-5}M ANF significantly enhanced the frequency of micronucleated cells per 500 binucleated lymphocytes. However, as shown in Tables 1 and 2, any of the five MSF-PCB congeners did not induce the formation of micronucleated cells by themselves and 3-MSF-2, 5, 2', 4', 5'-PenCB only significantly elevated the micronucleated cell rate in case of the simultaneous treatment with PenCDF. Based on the results of this study, the five MSF-PCB congeners examined including 3-MSF-4, 5, 3', 4'-TCB, which is probably the most effective AHH inhibitor among MSF-PCBs, are considered not to be an effective inducer of micronucleated cells in man, because their final concentrations used in this study were about 35,000 times higher than those in the lungs and adipose tissue of healthy Japanese people[8)].

The types of mutations which could contribute to spontaneous micronucleated cells include (a) mutations to kinetochore proteins, centromeres and spindle apparatus that could lead to unequal chromosome distribution or whole chromosome loss at anaphase, and (b) unrepaired DNA-strand breaks induced endogenously or as a result of exposure to environmental mutagens which may result in acentric chromosome fragments[4)]. Therefore, the assay of micronucleated cells can detect both clastogens and spindle poisons and can be preferentially used to estimate the dose of ionizing radiation or truly radiomimetic (non-S-dependent) chemicals to which people have been exposed.

According to the results of this study, the MSF-PCB congeners examined are considered not to be or very weak non-S-dependent genotoxic chemicals. We, however, have not investigated yet whether some MSF-PCBs are S-dependent genotoxic compounds or not. Sister chromatid exchanges (SCEs), which are not readily induced by ionizing radiation or non-S-dependent clastogens, have proved to be the most sensitive mammalian genotoxic endpoint for determining exposure to S-dependent chemicals, because SCEs occur during the S-phase of the cell cycle[27)], probably at DNA replication forks or sites where replication is incomplete[11)26)]. Consequently, in order to clarify whether some MSF-PCB congeners have the potency to elicit the genotoxicity such as S-dependent DNA insults, effects of the five MSF-PCBs on the induction of SCEs in cultured human lymphocytes are now under investigation in our laboratory.

References

1) Brown SS and Spudich JA: Cytochalasin inhibits the rate of elongation of actin filament fragments. J. Cell Biol. 83: 657-662, 1979.

2) Collman GW, Lundgren K, Shore D, Thompson CL and Lucier GW: Effects of α-naphthoflavone on levels of sister chromatid exchanges in lymphocytes from active and passive cigarette smokers: dose-response relationships. Cancer Res. 46: 6452-6455, 1986.

3) Countryman PI and Heddle JA: The production of micronuclei from chromosome aberrations in irradiated cultures of human lymphocytes. Mutation Res. 41: 321-332, 1976.

4) Fenech M: The cytokinesis-block micronucleus technique: a detailed description of the method and its application to genotoxicity studies in human populations. Mutation Res. 285: 35-44, 1993.

5) Fenech M and Morley AA: Measurement of micronuclei in lymphocytes. Mutation Res. 147: 29-36, 1985.

6) Haraguchi K, Kuroki H and Masuda Y: Polychlorinated biphenyl methylsulfone congeners in human tissues: identification of methylsulfonyl dichlorobiphenyls. Chemosphere 18: 477-484, 1989.

7) Haraguchi K, Kuroki H and Masuda Y: Synthesis and characterization of tissue-retainable methylsulfonyl polychlorinated biphenyl isomers. J. Agric. Fd Chem. 35: 178-182, 1987.

8) Haraguchi K, Kuroki H and Masuda Y: Capillary gas chromatographic analysis of methylsulphone metabolites of polychlorinated biphenyls retained in human tissues. J. Chromatogr. 361: 239-252, 1986.

9) Haraguchi K, Kuroki H, Masuda Y and Shigematsu N: Determination of methylthio and methylsulphone polychlorinated biphenyls in tissues of patients with 'Yusho'. Fd Chem. Toxicol. 22: 283-288, 1984.

10) Haraguchi K, Masuda Y, Kuroki H and Ohma M: Analysis of PCB-and PCT-methylsulfones in the blood of Yusho patients. Fukuoka Acta Med. 80: 291-295, 1989. (in Japanese)

11) Ishii Y and Bender MA: Effects of inhibitors of DNA synthesis on spontaneous and ultraviolet light-induced sister-chromatid exchanges in Chinese hamster cells. Mutation Res. 79: 19-32, 1980.

12) Lundgren K, Andries M, Thompson C and Lucier GW: Dioxin treatment of rats results in increased in vitro induction of sister chromatid exchanges by α-naphthoflavone: an animal model for human exposure to halogenated aromatics. Toxicol. Appl. Pharmacol. 85: 189-195, 1986.

13) Lundgren K, Collman GW, Wang-Wuu S, Tiernan T, Taylor M, Thompson CL and Lucier GW: Cytogenetic and chemical detection of human exposure to polyhalogenated aromatic hydrocarbons. Environ. Mol. Mutagen. 11: 1-11, 1988.

14) Lundgren K and Lucier GW: Differential enhancement of sister chromatid exchange frequencies by α-naphthoflavone in cultured lymphocytes from smokers and non-smokers. Mutation Res. 143: 131-135, 1985.

15) Nagayama J, Kiyohara C, Mohri N, Hirohata T, Haraguchi K and Masuda Y: Inhibitory effect of methylsulphonyl polychlorinated biphenyls on aryl hydrocarbon hydroxylase activity. Chemosphere 18: 701-708, 1989.

16) Nagayama J, Kiyohara C, Mohri N, Hirohata T, Haraguchi K and Masuda Y: Inhibitory effect of 3-methylsulphone-4, 5, 3', 4'-tetrachlorobiphenyl on aryl hydrocarbon hydroxylase activity. Fukuoka Acta Med. 78: 199-203, 1987. (in Japanese)

17) Nagayama J and Masuda Y: Effects of 3-methylsulphonyl-4, 5, 3', 4'-tetrachlorobiphenyl and 7, 8-benzoflavone on aryl hydrocarbon hydroxylase activities of murine hepatic microsomes prepared from inbred strains with different Ah responsiveness. Fukuoka Acta Med. 84: 195-202, 1993.

18) Nagayama J and Masuda Y: Effects of methylsulphonyl polychlorinated biphenyl homologues and 7,8-benzoflavone on aryl hydrocarbon hydroxylase activity. Fukuoka Acta Med. 84: 203-211, 1993.

19) Nagayama J, Mohri N, Haraguchi K, Masuda Y, Kiyohara C and Hirohata T: Effects of 3-methylsulphonyl-4, 5, 3', 4'-tetrachlorobiphenyl and 7, 8-benzoflavone on aryl hydrocarbon hydroxylase activity. Fukuoka Acta Med. 80: 201-209, 1989.

20) Nagayama J, Nagayama M, Haraguchi K, Kuroki H and Masuda Y: Effect of 2, 3, 4, 7, 8-pentachlorodibenzofuran and its analogues on induction of sister chromatid exchanges in cultured human lymphocytes. Fukuoka Acta Med. in press.

21) Nagayama J, Nagayama M, Iida T, Hirakawa H, Matsueda T and Masuda Y: Effects of highly toxic organochlorine compounds retained in human body on induction of sister chromatid exchanges in cultured human lymphocytes. Chemosphere 29: 2349-2354, 1994.

22) Nagayama J, Nagayama M and Masuda Y: Frequency of micronuclei induced in cultured lymphocytes by highly toxic organochlorine congeners. Fukuoka Acta Med. 84: 189-194, 1993.

23) Nagayama J, Nagayama M and Masuda Y: Genotoxicity of highly toxic organochlorine congeners in cultured human lymphocytes. Organohalo. Comp. 10: 165-168, 1992.

24) Nagayama J, Nagayama M, Wada K, Iida T, Hirakawa H, Matsueda T and Masuda Y: The effect of organochlorine compounds on the induction of sister chromatid exchanges in cultured human lymphocytes. Fukuoka Acta Med. 82: 221-227, 1991. (in Japanese)

25) Nagayama J, Wada K, Haraguchi K, Masuda Y and Handa S: Effects of 3-methylsulphonyl-4, 5, 3', 4'-tetrachlorobiphenyl and 7, 8-benzoflavone on aryl hydrocarbon hydroxylase activity in Ah responsive and Ah nonresponsive strains of mice. Fukuoka Acta Med. 82: 207-214, 1991. (in Japanese)

26) Painter RB: A replication model for sister-chromatid exchange, Mutation Res. 70: 337-341, 1980.

27) Wolff S, Bodycote J and Painter RB: Sister chromatid exchanges induced in Chinese hamster cells by UV irradiation of different stages of the cell cycle: the necessity for cells to pass through S. Mutation Res. 25: 73-81, 1974.

(Received for publication March 27, 1995)

(和文抄録)

サイトカイネシスブロック法によるメチルスルホニル PCBs 同族体のヒトリンパ球培養細胞の小核誘発性

九州大学医療技術短期大学部環境科学研究室
長山淳哉・長山真弓
第一薬科大学物理分析学・環境化学研究室
原口浩一・黒木広明・増田義人

カネミ油症患者や健常者の肺や血液は相対的に高濃度のメチルスルホニル PCBs (MSF-PCBs) により汚染されている．この研究では5種類の MSF-PCBs 同族体，つまり 3-メチルスルホニル-4, 5, 3', 4'-四塩化ビフェニール (3-MSF-4, 5, 3', 4'-TCB)，3-メチルスルホニル-4, 5, 2', 3'-四塩化ビフェニール (3-MSF-4, 5, 2', 3'-TCB)，3-メチルスルホニル-2, 5, 2', 4', 5'-五塩化ビフェニール (3-MSF-2, 5, 2', 4', 5'-PenCB)，4-メチルスルホニル-2, 5, 2', 3', 4'-五塩化ビフェニール (4-MSF-2, 5, 2', 3', 4'-PenCB) および 4-メチルスルホニル-2, 5, 2', 3', 5', 6'-六塩化ビフェニール (4-MSF-2, 5, 2', 3', 5', 6'-HCB) の非 S 依存型遺伝毒性を評価するために，ヒトリンパ球培養細胞の小核誘発性について研究した．また，カネミ油症の主要原因化学物質である 2, 3, 4, 7, 8-五塩化ダイベンゾフラン (PenCDF) や毒性が非常に高い 2, 3, 7, 8-四塩化ダイオキシン (TCDD)，3, 4, 5, 3', 4'-五塩化ビフェニール (Co-PenCB) が共存する場合の小核誘発性についても検討した．そして，次のような研究結果が得られた．1) ポジチブコントロール化学物質として用いた 7, 8-ベンゾフラボン (4×10^{-5}M) は有意な小核誘発性を示したが，5種類の MSF-PCBs 同族体は 5.2～9.6 ppm の濃度では，いずれも小核誘発性を示さなかった．2) いずれか1種類の MSF-PCBs 同族体と PenCDF (3.9 ppb)，TCDD (1.5 ppb) あるいは Co-PenCB (8.8 ppb) を同時にリンパ球培養細胞に処理した場合，3-MSF-2, 5, 2', 4', 5'-PenCB (5.2 ppm) と PenCDF 処理群でのみ有意に高い小核誘発性が観察された．

この研究で用いた MSF-PCBs 同族体の濃度は健常者の肺や脂肪組織のおよそ 35,000 倍も高い濃度であることから，実際には MSF-PCBs により小核誘発のような非 S 依存型遺伝毒性は生じないと考えられる．また，一般に，MSF-PCBs はこのタイプの遺伝毒性物質とはみなされないか，たとえそのような作用があるとしても非常に弱いと考えられる．

今後の課題として，MSF-PCBs の S 依存型遺伝毒性が研究される必要がある。S 依存型遺伝毒性の指標として，最も良く用いられるのは姉妹染色分体交換 (SCEs) 誘発性である．現在，私どもはこれら5種類の MSF-PCBs 同族体の SCEs 誘発性についても研究している．