Frequency of SCEs in Japanese Infants Exposed to Dioxins and PCBs through the Breast Milk

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Abstract Frequency of sister chromatid exchanges (SCEs), which has been considered an index to the synthetic and sharp genotoxic and clastogenic potencies, was examined by using the infant lymphocytes postnatal of around ten months, in order to evaluate the genotoxic or clastogenic potency of exposures to PCBs and dioxins through the breast milk. SCE frequency (mean±S.D.) as the control culture treated with the solvent, DMSO, alone (SCEcontrol) was 8.3±1.1/cell and that as the culture treated with 7,8-benzo-flavone (SCEANF) was 11.9±1.5/cell. In addition, the difference between SCEANF and SCEcontrol, that is, △ SCEs was calculated 3.6±1.5/cell.

The intake of PCBs (mean±S.D.) through the breast milk in these infants was 133±85 mg/kg body weight and that of dioxins 24±13 TEQ-ng/kg body weight. PCBs intake in the average was about 5,500 times greater than that of dioxins. We however, could not find any significant effect of PCBs on the frequencies of SCEs. Dioxins, on the contrary, showed a significant negative relationship to the frequency of △ SCEs. Therefore, exposure to dioxins through the breast milk seemed to elicit some genotoxic or clastogenic effects on Japanese general infants postnatal of around ten months.

Introduction

Foods in Japan have been polluted with some organochlorine pesticides, polychlorinated biphenyls (PCBs) and dioxins which are polychlorinated dibenzo- p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar PCBs (Co-PCBs) (1427). So, Japanese people have also been contaminated with these compounds (630). Consequently, PCBs and dioxins have been determined in Japanese breast milk and respective median concentrations of PCBs and dioxins on lipid weight basis were about 110 ppb and 24 ppt as the 2,3,7,8-tetrachlorodibenzo- p-dioxin toxic equivalent (2,3,7,8-TCDD TEQ) (1526). These results indicate that the median level of PCB was about 4,600 times higher than that of dioxins in Japanese breast milk. Therefore, we should give due attention to possible health effects of not only dioxins but also PCBs in Japanese infants.

In particular, prenatal and suckling stages of human life as well as animal life are considered to be the highest sensitivity to these hazardous chemicals, because of their remarkably fast differentiation and growth. We have already done some works from this viewpoint and got several worthwhile findings (1617). In the meantime, the formation mecha-
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nism of sister chromatid exchanges (SCEs) has not been well clarified yet at present. SCEs, however, seem to be a good index to the synthetic and sharp genotoxic and clastogenic potencies for several chemicals\(^1\)\(^2\)\(^3\)\(^9\). We have also been carried out several studies from this point of view and some important results have been obtained\(^2\)\(^3\)\(^4\)\(^5\)\(^6\). In this study, changes of SCE frequency in the lymphocytes of Japanese infants lactationally exposed to PCBs and dioxins were investigated, in order to evaluate their genotoxic and clastogenic potencies.

**Materials and Methods**

Fifty to 100 ml of breast milk at the postpartum period of 2 to 4 month were collected from 124 healthy mothers in total, mean age: 29 years old and the range: 22-41 years old, in July to October, 1994 and in June to October, 1995 and 1996. These samples of the breast milk were analyzed for PCBs by ECD gas chromatography method\(^1\)\(^2\)\(^3\)\(^6\)\(^7\)\(^8\) and for dioxins by HRGC-HRMS technique using a Finnigan MAT-95 mass spectrometer (Germany) directly interfaced with Varian Model 3400 gas chromatograph\(^9\)\(^1\)\(^0\)\(^1\)\(^1\).

Lymphocytes in the whole blood were stimulated with phytohemagglutinin and cultured for two replicative cycles in the presence of bromodeoxyuridine (100 mM) as detailed elsewhere\(^2\)\(^3\)\(^4\). Differential staining of sister chromatids was obtained by a fluorochrome plus Giemsa technique and the frequencies of control, solvent (DMSO) treated SCEs (SCEcontrol), 7,8-benzoflavone (ANF) treated SCEs (SCEANF) and SCEs (SCEANF - SCEcontrol) were evaluated.

In order to get normal distribution, frequencies of SCEcontrol, SCEANF and △SCEs and lactational exposure to PCBs and dioxins, namely, intakes of these compounds through the breast milk were transformed by a natural logarithm. Then, relationship between frequencies of SCEcontrol, SCEANF or △SCEs and the intakes of PCBs or dioxins was statistically examined by Spearman rank correlation method.

Toxic equivalent (TEQ) concentrations of the dioxins were calculated by using 1998 WHO TEF values\(^3\)\(^0\). TEQ-sum of all congeners of the dioxins determined in every breast milk sample was summarized as the total 2,3,7,8-TCDD TEQ concentration or level. Lactational exposure to PCBs or dioxins was estimated as a product of their respective daily intake, which was calculated with their respective level in the milk times an expected intake of breast milk in Japanese infant, that is, 120g/kg body weight, multiplied by individual duration (days) of breast feeding.

Five to 10 ml of the peripheral blood of 105 infants (60 males and 45 females with the mean age of 10 months old) born of these mothers were individually obtained by venipuncture in January ~ March of 1995, 1996 and 1997. Among them, SCE frequency of the lymphocytes was measured in 66 infants.

**Results**

1) SCE frequencies of the lymphocytes in the blood of Japanese infants

The frequency distributions of SCEcontrol, SCEANF and △SCEs of lymphocytes in the blood of infants are shown in Fig. 1. The mean value of SCEcontrol was 8.3/cell, and minimum and maximum ones were 6.5 and 12.4/cell, respectively. The average value of SCEANF was 11.9/cell, and the range was 9.1~15.6/cell. The mean of △SCE was 3.6/
Fig. 1 Frequency distributions of SCEcontrol, SCEANF and Δ SCEs in the lymphocytes of Japanese infants around ten months old
left: SCEcontrol, middle: SCEANF, right: Δ SCEs

cell, and respective values of minimum and maximum were 0.2 and 7.2/cell.

2) Intakes of PCBs and dioxins through the breast milk in Japanese infants

The distributions of total intakes of PCBs and dioxins in Japanese infants during breast-fed periods are indicated in Fig. 2. In case of PCBs, the average intake was 133 mg/kg body weight, and the lowest and highest ones were 28 and 388 mg/kg body weight, respectively. The mean intake of dioxins was 24 TEQ-ng/kg body weight with the range of 3.9 ~ 65 TEQ-ng/kg body weight. The average intake of PCBs was about 5,500 times greater than that of dioxins in Japanese infants of this study.

3) Relationships between lactational exposures to PCBs and dioxins and SCE frequencies of the lymphocytes

We could not find any correlation of the lactational exposures to PCBs with the frequencies of SCE control, SCEANF or Δ SCEs of the lymphocytes in the blood of Japanese infants.

In case of exposure to dioxins through the breast milk, dioxins did not show any signif-

Fig. 2 Distributions in total intakes of PCBs (left) and dioxins (right) through the breast milk in Japanese infants postnatal of around ten months.
Lactational exposures to dioxins & PCBs on SCEs

Fig. 3 Relationship between the exposure to dioxins through the breast milk and the frequency of SCEcontrol (left, $r=0.086$, $p=0.505$) or SCEANF (right, $r=-0.068$, $p=0.596$) of the lymphocytes in the blood of the Japanese infants postnatal of around ten months. Significant relationship with frequencies of SCEcontrol and SCEANF of the lymphocyte, as indicated in Fig. 3. Frequency of $\Delta$ SCEs, however, of the lymphocytes in the blood of infants postnatal of around 10 months was significantly decreased with the increasing lactational exposure to dioxins ($r=-0.292$, $p=0.020$), as shown in Fig. 4.

Discussion

Some conflicting results have been reported about the effect of aging on the frequency of SCEs. In some studies, it has been shown that the SCE frequency in human lymphocytes was significantly increased with an age$^{3,20-24}$, and in others not$^{10,13}$. In our studies, frequencies of both SCEcontrol and SCEANF in human lymphocytes at $20 \sim 64$-year-old people were clearly enhanced with age$^{21,24}$. In these investigations, average frequencies of SCEcontrol and SCEANF were 10.1 and 13.6/cell, respectively. In SCE frequency of this study, the former was 8.3/cell, and the latter 11.9/cell, which were apparently lower than the respective values in our previous studies. Based on these findings, it seems the SCE frequency probably increased with age in Japanese people.

In $0 \sim 75$-year-old persons, SCE frequency of the lymphocytes in the umbilical cord blood was the highest, that at $1 \sim 5$-year-old is the lowest and after these ages it increased with age$^2$. Further, we have to examine SCE frequency of the lymphocytes in umbilical cord blood or in the blood of newborn infant, in order to confirm their findings.

The estimated mean intake of PCBs was 133 mg/kg body weight and that of dioxins 24 TEQ-ng/kg body weight, which was four orders of magnitude lower than that of PCBs. In spite of such relatively large
amounts of intake, we have never observed the correlation of PCBs with any frequencies of SCEcontrol, SCEANF and △ SCEs in the lymphocyte of Japanese breast-fed infants postnatal of around 10 months. Contrastively, lactational exposure to dioxins was significantly and negatively correlated with the △ SCE frequency. At present, although we do not known the significance of this finding, the average intake of dioxins through the breast milk in Japanese general infants is about 30 times higher than the tolerable daily intake of dioxins in Japan, that is, 4 TEQ-pg/kg body weight\(^{25}\). Therefore, this level of intake of dioxins may cause some adverse health effects in sucklings, which are one of the most sensitive stages in not only humans but also animals to toxic chemicals. In adults, however, we could not find any significant effect of dioxin like chemicals, mostly PCDFs, on the frequencies of SCEcontrol, SCEANF and △ SCEs of the lymphocytes at the levels of more than 300 TEQ-pg/g on lipid weight basis in the blood, which was about 10 times higher than the Japanese normal contamination level of dioxins\(^{25}\). Accordingly, infants or sucklings are considered more sensitive to dioxins and dioxin like chemicals than adults.

This study, however, was carried out with rather small number of infants, so in order to get more conclusive findings for the effects of dioxins on SCE frequency and for the evaluation of their genotoxic and clastogenic potencies, further large-scale investigations are needed.

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乳児リンパ球細胞の姉妹染色分体交換頻度への授乳によるPCBsと\nダイオキシン類の影響

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姉妹染色分体交換（Sister chromatid exchanges, SCEs）はこれまで総合的な視角で細胞内での遺伝毒性または染色体傷害の指標として知られている。この研究では授乳によるPCBsとダイオキシン類への曝露と遺伝毒性の関係を調べる目的で、生後10ヶ月前後の一見健康な乳児の末梢血リンパ球細胞のSCEs頻度とPCBsやダイオキシン類への曝露との関係を統計学的に解析した。

溶媒のみ処理のコントロール群のSCEs頻度（SCEcontrol）（平均値±標準偏差，以下同様）は8.3±1.1/細胞であり，7,8-ペンゾフラボン（ANF）処理した場合のそれぞれ（SCEANF）は11.9±1.5/細胞であった。また両者の差，つまり，△SCEs（SCEANF-SCEcontrol）は3.6±1.5/細胞であった。また，これらの乳児のPCBsの母乳からの摂取量は133±65 mg/kg体重であり，ダイオキシン類では24±13 TEQ-ng/kg体重であった。したがって授乳によるPCBsの摂取量はダイオキシン類よりも約5,500倍多かった。

PCBへの授乳による乳児の曝露量はダイオキシン類よりもはるかに多いにもかかわらず，PCBsとSCEs頻度との関連性はSCEcontrol，SCEANFおよび△SCEsのいずれに対しても認められなかった。しかしダイオキシン類の場合には，統計的に有意でないけれども母乳からの摂取量が増えるにしたがってSCEcontrolが上昇し，また逆にSCEANFは減少する傾向を示した。その結果，△SCEsの有意な低下が観察された。

このようなSCEs頻度への影響がどのような臨床上の問題と関連するのか現時点では定かでないが，ダイオキシン類への授乳期の曝露が次世代に対しても影響をもたらすか否かの遺伝毒性あるいは染色体傷害作用をも有する可能性が示唆されたのであり，今後，さらに詳細な研究を行う必要がある。
Frequency of SCEs in Japanese Infants Lactationally Exposed to Organochlorone Pesticides

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Abstract Changes in the frequency of sister chromatid exchanges (SCEs) in cultured mammalian cells have been considered as indices to the synthetic and sharp genotoxic and clastogenic potencies. Frequency of SCEs in cultured lymphocyte obtained from infants postnatal of around ten months was examined to evaluate the genotoxic or clastogenic potency of lactational exposures to some organochlorine pesticides such as HCHs, DDT and chlordane in this study.

Frequency (median, min. ～ max.) of SCEs as the control culture treated with the solvent, DMSO, alone (SCE control) was 8.0/cell, 6.5 ～12.4/cell, that as the culture treated with 7,8-benzoflavone (SCEANF) was 11.8/cell, 9.1 ～ 15.6/cell and the difference between SCEANF and SCEcontrol, that is, Δ SCEs was 3.9/cell, 0.2 ～ 7.2/cell. Lactational exposures (median, min. ～ max.) to the three organochlorine pesticides were as follows: HCHs; 341 mg/kg body weight, 43 ～ 1449 mg/kg body weight, DDT; 272 mg/kg body weight, 33 ～ 1361 mg/kg body weight and chlordane; 69 mg/kg body weight, 13 ～ 379 mg/kg body weight.

The median exposure level to chlordane through the breast milk was one fourth to one fifth times lower than that to HCHs or DDT and probably due to such small amounts of it’s intake, we could not find any correlation of chlordane with frequencies of SCEcontrol, SCEANF and Δ SCEs in cultured lymphocytes of Japanese infants postnatal of about ten months. In the meantime, frequencies of SCEcontrol and SCEANF showed increasing and decreasing tendencies with the increasing exposure to DDT and also HCHs through the breast milk. In consequence, the frequency of Δ SCEs was significantly lowered. Therefore, lactational exposure to DDT and HCHs seemed to elicit some genotoxic or clastogenic effects on Japanese general infants at ages of around the months. These compounds were also considered rather potent S-dependent clastogens than non-S-dependent ones. So, further detailed studies are needed not only to get conclusive findings but also from this viewpoint.

Introduction

Our environments including food have been polluted with some organochlorine compounds such as polychlorinated dibenzo- p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs) and pesticides\(^\text{[1127]}\). Accordingly, Japanese people have also been contaminated with these compounds\(^\text{[380]}\). Some pesticides such as hexachlorocyclohexans (HCHs), 1,1,1-trichloro-2, 2-bis- (4-chlorophenyl) -ethane (DDT), dieldrin, heptachlor epoxide (HCE) and chlor-
Lactational exposures to DDT & HCHs on SCEs

Dane have been determined in Japanese breast milk\textsuperscript{4,12,13,14,15,24}. Their levels in the breast milk were more than 100 to 10,000 times higher than those of PCDDs and PCDFs, which were so-called dioxins, in 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) toxic equivalent (TEQ) value, as indicated in the above mentioned studies. Therefore, we should give due attention to possible health consequences of these pesticides as well as PCDDs and PCDFs. In particular, prenatal and suckling stages of not only humans but also animals are considered the highest sensitivity to these compounds, because of their remarkably rapid differentiation and growth.

In the meantime, cytogenetic changes such as micronucleated cells (MNs) and sister chromatid exchanges (SCEs) have frequently been utilized as indicators of mostly genetic damage due to exposure to different carcinogens or mutagens. These two cytogenetic changes are considered to occur as results of different mechanisms of DNA or chromosome damage. MNs have been considered to be the result of chromosomes lagging behind the genome at cell division. On the other hand, SCEs are formed during the S phase after an initial change in the form of DNA base damage\textsuperscript{28}, when quadriradials as mitotic chiasmas are a consequence of mitotic crossing over\textsuperscript{30}. Changes in frequency of MNs and SCEs have been considered good indices to the synthetic and sharp genotoxic or clastogenic potency for several chemicals\textsuperscript{7,8,26,29}. We have already carried out several studies from this viewpoint and some valuable results have been obtained\textsuperscript{17,18,19,21,23,24}.

In this study, changes of SCEs frequency in cultured lymphocytes of Japanese infants exposed to HCHs, DDT and chlordane through the breast milk were investigated, in order to evaluate their genotoxic and clastogenic potencies.

**Materials and Methods**

Fifty to 100 ml of breast milk at the postpartum period of 2nd to 4th month were collected from 124 healthy mothers in total, mean age: 29 years old and the range: 22 ~ 41 years old, in July to October, 1994 and in June to October, 1995 and 1996. These samples of the breast milk were analyzed for HCHs, DDT and chlordane by ECD gas chromatography method\textsuperscript{11,12,13,14,15,24}.

Lactational exposure to these pesticides was estimated as a product of their respective daily intake, which was calculated with their respective level in the milk times an expected intake of breast milk in Japanese infants, that is, 120g/kg body weight, multiplied by individual duration (days) of breast feeding.

Five to 10 ml of the peripheral blood of 105 infants (60 males and 45 females with the mean age of 10 months old) born of these mothers were individually obtained by venipuncture in January ~ March of 1995, 1996 and 1997. Among them, SCE frequency of the lymphocytes was measured in 63 infants.

Lymphocytes in the whole blood were stimulated with phytohemagglutinin and cultured for two replicative cycles in the presence of bromodeoxyuridine (100 mM) as detailed elsewhere\textsuperscript{17,18,19,23}. Differential staining of sister chromatids was obtained by a fluorochrome plus Giemsa technique and the frequencies of control, solvent (DMSO) treated SCEs (SCEcontrol), 7,8-benzoflavone (ANF) treated SCEs (SCEANF) and Δ SCEs (SCEANF – SCEcontrol) were evaluated.

In order to get normal distribution, fre-
quencies of SCEcontrol, SCEANF and △ SCEs and lactational exposures to HCHs, DDT and chlordane, namely, intakes of these compounds through the breast milk were transformed by a natural logarithm. Then, relationship between SCEcontrol, SCEANF or △ SCEs and the intakes of the organochlorine pesticides was statistically examined by Spearman rank correlation method.

**Results**

The distributions in the total intakes of HCHs, DDT and chlordane in Japanese infants during breast-fed periods are shown in Fig. 1. Total intakes (median, min. ~ max.) of HCHs (mostly β-HCH), DDT (sum of p, p'-DDE and p, p'-DDT) and chlordane (sum of oxychlordane, trans-nonachlor and cis-nonachlor) were as follows: HCHs; 341 mg/kg body weight, 43 ~ 1449 mg/kg body weight, DDT; 272 mg/kg body weight, 33 ~ 1361 mg/kg body weight and chlordane; 69 mg/kg body weight, 13 ~ 379 mg/kg body weight. Median intake of chlordane were one order of magnitude lower than that of HCHs or DDT.

Frequencies (median, min. ~ max.) of SCEcontrol, SCEANF and △ SCEs of cultured lymphocytes in the blood of 63 Japanese breast-fed infants were 8.0/cell, 6.5 ~ 12.4/cell, 11.8/cell, 9.1 ~ 15.6/cell and 3.9/cell, 0.2 ~ 7.2/cell, respectively.

We could not find any significant relationship between the lactational exposure to chlordane and the frequency of SCEcontrol, SCEANF or △ SCEs. Significant correlations were not observed either between the frequency of SCEcontrol or SCEANF and the intake of HCHs or DDT. Fig. 2 indicates the correlations of the lactational exposure to DDT with the frequency of SCEcontrol or SCEANF. We, however, could see that the frequency of △ SCEs of lymphocytes in the blood of infants postnatal of around ten months was significantly decreased with the increasing intake of DDT through the breast milk (r = -0.247, p = 0.050), as shown in Fig. 3. This kind of relationship was also observed between the exposure to HCHs and the frequency of △ SCEs (r = -0.274, p = 0.065), which was indicated in Fig. 4.
Lactational exposures to DDT & HCHs on SCEs

Discussion

The estimated median intake of chlordane was 69 mg/kg body weight, which was one fourth to one fifth of that of HCHs or DDT. Probably due to such small amounts of intake of chlordane through the breast milk, we could not observe any correlation of chlordane with frequencies of SCEcontrol, SCEANF and ΔSCEs in cultured lymphocytes of Japanese infants postnatal of around ten months.

Figs. 2 and 3 show relationship between the lactational exposure to DDT and the frequency of SCEcontrol, SCEANF or ΔSCEs. Frequencies of SCEcontrol and SCEANF showed increasing and decreasing tendencies with the increasing intake of DDT through the breast milk, respectively. Same kinds of tendencies were also seen in HCHs of this study and in case of dioxins 16). Increasing exposures to DDT, HCHs and dioxins through the breast milk significantly lowered the frequency of ΔSCEs, as indicated in Figs. 3 and 4, and our paper16). Frequency of ΔSCEs has been considered more sensitive biomarker than that of SCEcontrol or SCEANF in the evaluation of...
genotoxic and clastogenic potencies\textsuperscript{10})\textsuperscript{10}. Although we do not know the significance of these findings at present, lactational exposures to these organochlorine compounds seem to elicit some genotoxic or clastogenic effects in Japanese general infants postnatal of around ten months.

The types of mutations which could contribute to spontaneous MNs 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\textsuperscript{8}). Therefore, the assay of MNs can detect both clastogens and spindle poisons and can be preferentially used to estimate the dose of ionizing radiations or truly radiomimetic (non-S-dependent) chemicals to which people have been exposed. On the other hand, SCEs, which are not readily induced by ionizing radiations or non-S-dependent clastogens, have proved to be the most sensitive mammalian genotoxic endpoint for determining exposure to S-dependent chemicals, because SCEs are considered to form during the S-phase of the cell cycle\textsuperscript{8}), probably at DNA replication forks or sites where replication is incomplete\textsuperscript{6,8}). Therefore, according to the results of this study and our other investigations\textsuperscript{10,22}), DDT, HCHs and dioxins seemed rather potent S-dependent clastogens than non-S-dependent ones.

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乳児リンパ球細胞の姊妹染色分体交換頻度への授乳による農薬の影響

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総合的で鈍敏な遺伝毒性指標として知られている姊妹染色分体交換（Sister chromatid exchanges, SCEs）頻度を用いて、授乳によるHCHs, DDT およびクロルディンへの曝露と乳児リンパ球細胞のSCEs頻度との関係を数理統計学的に調べた。

リンパ球細胞の観察中に溶媒（DMSO）のみ処理したコントロール群のSCEs頻度すなわちSCEcontrol（中央値、最小値～最大値、以下同様）は8.0/細胞、6.5～12.4/細胞、7.8−ベンゾピラボン（ANF）処理した場合のSCEs頻度SCEANFは11.8/細胞、9.1～15.6/細胞、また両者の差SCEANF−SCEcontrol、△SCEsは3.9/細胞、0.2～7.2/細胞であった。母乳から有機塩素系農薬の摂取量は次のようである。HCHs: 341 mg/kg体重、43～1449 mg/kg 体重、DDT: 272 mg/kg 体重、33～1361 mg/kg 体重、クロルディン: 69 mg/kg 体重、13～379 mg/kg 体重。

クロルディンの授乳による曝露量は中央値で比較するとHCHsとDDTの4分の1から5分の1程度と少なく、SCEs 頻度との関係も認められなかった。一方、HCHsとDDTについては母乳からこれらの農薬の摂取量が増加するにつれて有意ではなく、けれどもSCEcontrolが上昇し、逆にSCEANFが減少する傾向が観察された。その結果、いずれの農薬でも△SCEs 頻度の有意な低下が認められた。このようなSCEsへの影響が臨床上どのような意義があるのか不明であるが、HCHsやDDTへの授乳期の曝露が次世代に対して何らかの遺伝毒性作用を及ぼす可能性が示唆されたのであり、今後、さらに詳細な研究が必要である。また同様の結果は母乳からのダイオキシン類への曝露でも観察されているが、SCEsへの影響は細胞分裂のS期依存性遺伝毒性物質とみなされ、この視点からの研究も重要である。