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Chromosome analyses in dairy cows exposed to dioxins and dioxin-like PCBs using the SCE test

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ABSTRACT - Peripheral blood cultures were performed to study the sister chromatid exchanges (SCE) in samples from 15 cows (Valdostana x Piedmontese crossbreds) showing average milk values of dioxins+furans+PCBs higher (18.56 pg/g of fat as WHO-TEQ) than those permitted (6.0 pg/g of dioxins+furans+PCBs as WHO-TEQ) and the results were compared with samples from 16 Valdostana dairy cows (1.75 pg/g of fat as WHO-TEQ) used as control. Significant ($P<0.01$) higher mean number of SCE/cell (7.10±2.8) were found in cows showing higher levels of dioxins and PCB compared to those achieved in the controls (SCE/cell=5.24±2.51).

Key words: Cattle, Chromosome, Dioxin, SCE-test.

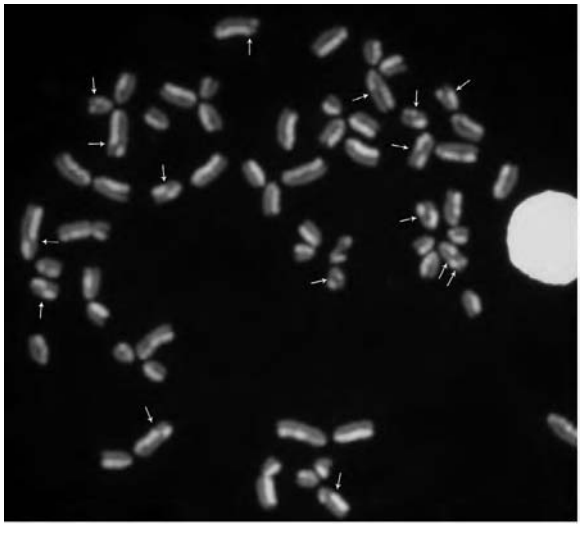
Introduction - Chromatin is the main component of chromosomes. It is dispersed in the interphase nucleus to make easier the DNA duplication and highly compacted (1,000x) at the metaphase stage to equally distribute the genome in the forming cells. This complex process, which takes place in about 15-18 h in eukaryotic cells, is always exposed to possible damages due to the presence of environmental mutagens (Tucker and Preston, 1996; Pfeiffer *et al*., 2000, Mahata *et al*., 2002; Deckbar *et al*., 2006; Payne *et al*., 2008). Polychlorodibenzodioxins (PCDDs) and related substances including polychlorodibenzofurans (PCDF), and the dioxin-like polychlorobiphenyls (DL-PCBs) are powerful mutagens that can enter the food chain as the result of a number of industrial processes or of waste incineration. When cellular defenses are overwhelmed, damages at the chromosomal level may occur. So, cytogenetic tests could be useful to reveal the presence of such mutagens in the food chain by simply monitoring food producing species (Iannuzzi *et al*., 2004; Perucatti *et al*., 2006). Indeed, since PCDDs and DL-PCBs are characterized by a very long biological half-life and may therefore build-up to a significant extent in livestock, animal productions represent the main source of the exposure to such compounds for humans. Few cytogenetic studies performed in both humans and animals exposed *in vivo* to PCDDs, in particular to tetrachlorodibenzo-p-dioxin (TCDD), gave contradictory results (see review in Iannuzzi *et al*., 2004).

Two main cytogenetic tests are applied to measure damages in chromosomes: the test of chromosome abnormalities (AC) (gap, chromatid and chromosomes breaks, fragments, aneuploidy) and the test of the "Sister Chromatid Exchange" (SCE). These tests are applied separately or simultaneously to both animals potentially exposed to mutagens and those which are not exposed (control). Base-

line levels of SCEs have been established for several species (see review in Peretti *et al*., 2008). The higher the values of these tests (AC or SCE), the higher the chromosome fragility and the likelihood of cell mutations are. Indeed, it is possible that some unbalanced gametes may be generated when the chromosome fragility is high resulting in the formation of unbalanced embryos which can die in early embryonic life. The female thereafter returns to **oestrus but with delay, with reduction of its fertility** value. In the present study, we report the preliminary results of SCE-values found in cows exposed to relatively high and low (control) levels of dioxin-like PCBs.

Material and methods - Thirty-one dairy cows were used throughout the study. Fifteen of them (group A) were hybrids from Valdostana x Piedmontese reared in a farm of Northern Italy showing levels of PCCD+PCDF+DL-PCB in the milk mass higher (18.56 pg/g of fat as WHO-TEQ) than those permitted (6.0 pg/g of fat as WHO-TEQ); another group (B) of 16 individuals (Valdostana breed) was from a farm located in the same region showing very low milk mass WHO-TEQ values (1.75 pg/g of fat) and was therefore used as control. Peripheral blood samples were cultured for 72 h in RPMI TC-medium enriched with bovine calf serum (10%), penicillin-streptomicyn (1%) and Concanavalin A (15 µg/ml) as mitogen. Bromodeoxyuridine (BrdU, 10 µg/ml) was added 28 h before harvesting the cells, which were subsequently treated with colcemid for the last 1.5 h, then with an hypotonic solution and three fixations in methanol-acetic acid (3:1), and finally fixed on cleaned, wet and cold slides. Slides were stained with acridine orange (0.01%) for 10 min, washed in tap and distilled water, and air dried. Then slides were mounted in phosphate buffer under sealed coverlisps, and observed a day or more after, under a fluorescence microscope. About 35 metaphases were studied for each animal. All images were captured with cameras connected to microscopes and later processed by counting the number of SCEs for each metaphase so to obtain the mean value of SCEs for each animal and group of animals. Statistical analyses were performed using the T-student test.

Figure 1. Female cattle metaphase plate treated for the SCE-test. Note the clear differential staining between sister chromatids and the presence of several SCEs (arrows).



Results and conclusions - Mean values of SCE/cell (Figure 1) in cows showing higher and lower levels of PCDD, PCDF, and DL-PCBs than those permitted, respectively, are reported in Table 1. As compared to controls (5.24±2.51), significant ($P<0.01$) higher mean values of SCEs (7.10±2.89) were found in group A cows, which exhibit abnormal values only for DL-PCBs (16.09 pg/g on a total of 18.56 pg/g as sum of PCDD, PCDF and DL-PCBs). Similar results were obtained in sheep exposed to dioxins during pasturage (Iannuzzi *et al*., 2004; Perucatti *et al*., 2006) and in river buffaloes affected by limb malformations (Peretti *et al.,* 2008).

Since an increase in SCE values has been reported as the result of the exposure to many other environmental chemicals, further research is warranted involving a larger number of animals and more specific tests (e.g. expression profile of target genes) to confirm the main involvement of PCDD, PCDF and DL-PCBs in the cytogenetic alterations observed in the present study.

Table 1. Number of SCEs in cattle exposed to relatively high and low (control) levels of dioxins and PCBs; permitted values in brackets. Group B was considered as control.

Animals (n)	PCDD+PCDF (WHO-TEQ) pg/g of fat	DL-PCB (WHO-TEQ) pg/g of fat	PCCD+PCDF+DL-PCB (WHO-TEQ) pg/g of fat	SCE/cell mean±sd
Group A (15)	1.66 (3.0)	16.09 (3.0)	18.56 (6.0)	7.10±2.89[a]
Group B (16)	1.20 (3.0)	0.55 (3.0)	1.75 (6.0)	5.24±2.51

[a]*P<0.01.*

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REFERENCES - **Deckbar**, D., Birraux, J., Krempler, A., Tchouandong, L., Beucher, A., Walker, S., Stiff, T., Jeggo, P., Lobrich, M., 2006. Chromosome breakage after G2 checkpoint release. J. Cell Biol. 176:749-755. **Iannuzzi**, L., Perucatti, A., Di Meo, G.P., Polimeno, F., Ciotola, F., Incarnato, D., Peretti, V., Caputi-Jambrenghi, A., Pecoraro, A., Manniti, F., D'Alessandro, A., Vonghia, G., 2004. Chromosome fragility in two sheep flocks exposed to dioxins during pasturage. Mutagenesis. 19:355-359. **Mahata**, J., Basu, A., Ghoshal, S., Sarkar, J.N., Roy, A.K., Poddar, G., Nandy, A.K., Banerjee A., Ray, K., Natarajan, A.T., Nilsson, R., Giri, A.K., 2002. Chromosomal aberrations and sister chromatid exchanges in individuals exposed to arsenic through drinking water in West Bengal, India. Mut. Res. 534:133-143. **Payne**, S.R., Zhang, S., Tsuchiya, K., Moser, R., Gurley, K.E., Longton, G., deBoer, J., Kemp, C.J., 2008. $P27^{kip1}$ deficiency impairs G_2/M arrest in response to DNA damage, leading to an increase in genetic instability. Mol. Cell. Biol. 28:536-537. **Peretti**, V., Ciotola, F., Albarella, S., Restucci, B., Meomartino, L., Ferretti, L., Barbieri, V., Iannuzzi, L., 2008. Increased SCE levels in Mediterranean Italian buffaloes affected by limb malformation (transversal hemimelia). Cytogenet. Genome Res. 120:183-187. **Perucatti**, A., Di Meo, G.P., Albarella, S., Ciotola, F., Incarnato, D., Caputi Jambrenghi, A., Peretti, V., Vonghia, G., Iannuzzi, L., 2006. Increased frequencies of both chromosome abnormalities and SCEs in two sheep flocks exposed to high dioxin levels during pasturage. Mutagenesis. 21:67-75. **Pfeiffer**, P., Goedecke, W., Obe, G., 2000. Mechanism of DNA doublestrand break repair and their potential to induce chromosomal aberrations. Mutagenesis. 15:289-302. **Tucker**, J.D., Preston, R.J., 1996. Chromosome aberrations, micronuclei, aneuploidy, sister chromatid exchanges, and cancer risk assessment. Mut. Res. 365:147-159.