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Chromosome fragility in two sheep flocks exposed to dioxins during pasturage

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In the last 3 years several farms raising cattle, river buffalo and sheep have been unable to sell dairy milk due to the presence of high levels of dioxins. Furthermore, several cases of abortion (around 25% of total births) and abnormal foetuses (2.5% of total births) were recorded in two flocks of sheep raised in the province of Naples where a higher level of dioxins (5.27 pg/g fat, as human WHO TCDD equivalent) have been found in the milk mass than that permitted (3.0 pg/g fat, as human WHO TCDD equivalent). Cytogenetic investigations were carried out on 24 sheep (all females), randomly sampled from the two different flocks, one abnormal foetus and 11 female sheep (control) raised ~80 km from the area where the two exposed flocks were raised. Frequencies of aneuploid cells, gaps, chromatid breaks, chromosome breaks, fragments and sister chromatid exchange (SCE) were determined. While no differences were observed between the number of aneuploid cells (15% of total cell population) of both exposed animals and controls, significant ($P < 0.001$) increases in the frequencies of other chromosome abnormalities (mean chromosome abnormality/cell = 0.76 ± 1.1) and SCEs (mean SCE/cell = 9.4 ± 3.7) were found in the exposed animals, compared with the control (mean chromosome abnormality/cell = 0.18 ± 0.4; mean SCE/cell = 7.1 ± 3.0). Significantly higher values of SCEs (mean SCE/cell = 10.9 ± 4.4) were also found in the abnormal foetus compared with the control. Chemical analyses on soil, grass and water at two sites where the two flocks were pastured established that doses of dioxins (17 different types) were below the legally permitted limits.

Introduction

Dioxins belong to a large family of halogenated aromatic hydrocarbons widespread in the environment and known to be mutagenic. Dioxins do not occur naturally but are produced when manufacturing some herbicides (i.e. 2,4,5-T), germicides (i.e. hexachlorophene), pulp and paper, as well as during the combustion of wood in the presence of chlorine, by fires involving chlorinated benzenes and biphenyls and by municipal waste incinerators. Some possible sources of dioxins, like paper bleaching, seem to be noticeably reduced or eliminated by new bleaching technologies (Jorling, 2000). The best-known dioxin contaminant is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), which has various toxic effects on both the liver and immune system with evident teratogenic effects in both humans (Manz *et al*., 1991; McGregor *et al*., 1998; Bertazzi *et al*., 2001; Rier and Foster, 2002; Pesatori *et al*., 2003) and mice (Chapman and Schiller, 1985; Kociba, 1991).

TCDD is bio-accumulated in the food chain as it has a long half-life in the body, varying between 7 and 11 years (Pirkle *et al*., 1989; Wolfe *et al*., 1994). Being lipophilic, TCDD accumulates in the fat of animals and fish, especially in milk.

The few cytogenetic studies performed in both humans and animals exposed *in vivo* to dioxins, in particular to TCDD, have given contradictory results. Indeed, Kaye *et al*. (1985) found a significant increase in chromosome breakage in males exposed to agent orange and TCDD, compared with both unexposed males and their children, while sister chromatid exchange (SCE) frequency was not increased. Tenchini *et al*. (1983) found large increases in the frequency of both aberrant cells and in the average number of chromosome aberrations per damaged cell in foetal tissues after exposure during pregnancy (compared with the control), but the frequency of chromosome abnormalities did not differ from the control when studying the maternal blood and placenta. No statistical differences were observed in either chromosome aberrations or SCEs between humans exposed to TCDD (exceeding 40 parts per trillion in blood lipid) and unexposed humans of similar age (Zober *et al*., 1993). No cytogenetic effects were observed in mouse liver cells after exposure to TCDD, although high doses of TCDD were the cause of hepatocellular necrosis (Brook *et al*., 1988). No significant differences were noted, compared with the control, in the levels of SCE in cells of rhesus monkeys exposed *in vitro* to TCDD (Lim *et al*., 1987). No chromosome aberrations were found in human spermatozoa *in vitro* exposed to several chemicals, including dioxin (Kamiguchi and Tateno, 2002).

In Italy, several epidemiological studies following the 'Seveso accident' (1976) revealed increased risk for several types of cancers in humans (Pesatori *et al*., 2003), especially in men (Bertazzi *et al*., 2001). However, no systematic studies have been undertaken in livestock.

In south Italy various chemical analyses performed during the last three years in milk of cattle, river buffalo and sheep raised on several farms in the provinces of Naples and Caserta (Campania) revealed the presence of dioxins at higher doses than those permitted (3 pg/g fat, as human WHO TCDD equivalent). Hence, several farms have not been able to sell (or process) their milk, which was sequestered by the local health authorities and destroyed. So, while consumers are adequately protected from possible intake of large amounts of dioxins, farmers have incurred severe economic damage, only

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partially reimbursed by the Campania region, which is now considering the slaughter of about 7000 animals and reimbursement of head values.

On two farms where sheep (*Ovis aries*, 2n = 54) are raised and high doses of dioxins (human WHO TCDD equivalent = 5.27 pg/g fat) were found in the milk, high numbers of abortions, probably due to the high percentage of animals positive for *Brucella abortis*, and several abnormal foetuses (on total number of births) were recorded by both breeders and the local health authorities. Twenty-four females randomly selected, except for three females which had abnormal foetuses, and one abnormal foetus were tested cytogenetically for the presence of chromosomal fragility in these animals, compared with sheep (control) living in an area far from that where the animals were exposed to dioxins during pasturage.

In the present study we report the preliminary results of our investigation as significant increases in both chromosome abnormalities and SCEs found in the exposed animals, compared with the controls, revealing an increase in chromosome fragility in the cells of animals exposed to dioxins during pasturage. To our knowledge, this is the first report of chromosome fragility and abnormal foetuses in livestock exposed to dioxins.

Materials and methods

Sheep herds

The two flocks each numbered ~200 head, of which about 100 females are normally lactating. They were raised on two different farms in Naples province, Brusciano municipality. The animals were hybrids of different breeds, of which one (Comisana) was predominant. The sheep we used as controls were Comisana, raised in the province of Benevento, ~80 km from the area where the two exposed flocks were raised. The age of both the exposed animals and controls varied from 1 to 4 years. The animals were fed only on pasture, which was often the residue of various vegetable crops grown in the province of Naples. The animals used as controls were only fed natural pasture. Percentages of abortions and abnormal foetuses were calculated on total births/year.

Chemical analyses

Chemical analyses to check for the presence of dioxins in the milk mass, soil, grass and water were performed by a specialized laboratory as requested by the local health authorities. Samples of milk mass were from the two flocks, while samples of soil, grass and water were from two sites where the two flocks were pastured. Seventeen different types of dioxins (grouped in dioxins and furans) were determined.

Cell cultures

Twenty-four randomly selected ewes (with the exception of three with abnormal foetuses) raised on two farms in the province of Naples and 11 ewes (as control) raised in the province of Benevento (~80 km from the area where the two flocks were raised) were investigated cytogenetically.

Peripheral blood samples from both exposed animals and controls were cultured for 48 h at 37.8°C in McCoy's 5A modified TC medium (Gibco-BRL), foetal calf serum (10%), penicillin–streptomycin (1%) and concanavalin A (15 μg/ml) as mitogen. Two types of cell cultures, without (normal cultures) and with addition of 5-bromodeoxyuridine (BrdU) (10 μg/ml) 30 h before harvesting, for incorporation into DNA during the last two cell cycles (SCE test), were performed. Cells from both types of cell culture were harvested after exactly 48 h, including a colcemid (0.5 μg/ml) treatment for 1.5 h. A hypotonic treatment (20 min at the 37.5°C) and three fixations (the third overnight) in methanol–acetic acid (3:1) followed.

We tried to obtain primary fibroblast cell cultures from the three abnormal foetuses. Unfortunately, only one sample (case 1) underwent cell proliferation and could be investigated by the SCE test. As for the blood cultures, BrdU (10 μg/ml) was added 30 h before harvesting in the SCE test.

Cell suspensions from both blood and fibroblast cell cultures were dropped onto cleaned, wet, cold slides and stored in slide boxes. Slides from both cultures A and B were stained with acridine orange (0.01%) for 10 min, washed in tap and distilled water, air dried, mounted after 30 min in phosphate buffer (pH 7.0) and sealed with rubber cement. Slides were observed 1 day later under two fluorescent microscopes connected to CCD cameras (Sensys and Coolsnap CF; Photometrics).

For each animal at least 50 cells were examined from slides of normal cultures (aneuploidy, gaps, chromatid breaks, chromosome breaks and fragments) and 25 for BrdU-treated cells (SCE test), respectively. For the SCE test only cells at the second cycle of replication in the presence of BrdU and with a complete chromosome set (2n = 54) were considered. All metaphases, captured by two CCD cameras, were simultaneously studied by three cytogeneticists.

Statistical analysis

An independent two-sample Student's *t*-test was performed and a confidence interval generated to compare the two groups of animals by determining whether or not there is any evidence that the differences among three cytogenetic tests we applied: aneuploidy, chromosome abnormalities (gaps, chromatid breaks, chromosome breaks and fragments) and SCE were other than 0. The 95% confidence interval for the difference in cell population means are (−0.8, −0.4), (–0.7, −0.5) and (−2.7, −1.7) for aneuploidy, chromosome abnormalities and SCEs, respectively. The hypothesis test statistics all had a P value of 0.001, thus there is evidence for a difference between animals exposed to dioxin versus animals not exposed.

Results

Chemical analyses

Table I shows that levels of dioxins were higher in the milk mass and lower in soil, grass and water than those permitted.

Abortions and abnormal foetuses

Abortions among total births of the two exposed flocks were 24.5% and 25% in 2002 and 2003 (until October 30), respectively, while abnormal foetuses were 2.4% and 2.5% in 2002 and 2003 (until October 30), respectively (Table II). These values were appreciably higher than those observed in the controls. However, analyses performed in blood samples (~50% of animals) revealed the presence of *Brucella abortis* on 52% of animals.

Table I. Results of the chemical analyses searching for 17 types of dioxins (grouped in dioxins+furans and PCBs and expressed as 'human WHO TCDD equivalents') on milk mass of two sheep herds, as well as on soil, grass and water of two sites where sheep are pastured

Source	Dioxins + furans (WHO TE pg/g)	PCBs (WHO TE pg/g)
Milk mass	5.27 (3.0)	
Soil[a]	1.2182 (10.0)	0.2750 (1.0)
Grass[a]	0.1589 (0.75)	0.1075 (0.75)
Water[a]	0.0714 (4.0)	0.2 (10.0)

Reported permitted maximum values are shown in parentheses.
[a]Mean values between the two sites.

Table II. Number of births, abortions and abnormal foetuses recorded in 2002 and 2003 (until October 30) in the exposed and control herds

Herd	Births 2002 (*n*)	Births 2003 (*n*)	Abortions 2002 (*n*) (%)	Abortions 2003 (*n*) (%)	Abnormal foetuses 2002 (*n*) (%)	Abnormal foetuses 2003 (*n*) (%)
Exposed	208	200	51 (24.5)	50 (25)	5 (2.4)	5 (2.5)
Control	220	215	2 (0.9)	2 (0.9)		

The three abnormal foetuses examined showed abnormalities as follows: case 1, complete lack of anterior half with reduction of posterior limbs; case 2, body apparently normal but with complete lack of skull and with ears directly joined to the neck; case 3, body entirely deformed with short anterior limbs.

Cytogenetic analysis

The percentage of abnormal cells (aneuploidy, gaps, chromatid breaks, chromosome breaks and fragments) was considerably higher in the exposed animals (52.3%) than that (28.3%) achieved in the control (Table III). Table IV shows the percentages of chromosome abnormalities with reference to both abnormal and total cells examined. While the percentages of aneuploid cells (all hypoploids) were similar in both exposed animals (15.4%) and controls (15.3%) when total number of cells examined was considered (Table IV), the remaining percentages related to gaps, chromatid breaks, chromosome breaks and fragments were considerably higher in both abnormal and total cells of exposed flocks when compared with those of the controls (Table IV). Gaps are the most common chromosome abnormality found in the exposed flocks, followed by aneuploidy, chromatid breaks, chromosome breaks and fragments (Table IV), in contrast to the control where the highest percentage was achieved in aneuploid cells (Table IV). The number of chromosome abnormalities (gaps, chromatid breaks, chromosome breaks and fragments) was considerably higher (>4-fold) in both diploid and aneuploid cells of exposed animals, compared with those observed in the controls, these differences being highly significant ($P < 0.001$) (Table V).

Similar results were obtained in the SCE test (Table VI). Indeed, a significant increase ($P < 0.001$) in the mean number of SCEs was observed in the exposed group of animals (9.4 ± 3.7 SCE/cell), compared with that of the control (7.1 ± 3.0 SCE/cell). A significantly increased number of SCEs (10.9 ± 4.4 SCE/cell, $P < 0.001$) was also found in the cells from an abnormal foetus (case 1) when compared with the controls, although SCE analyses of foetus were performed on fibroblasts and those of controls on lymphocytes. No statistically significant differences were found between the mean number of SCE per cell from the abnormal foetus and exposed animals.

Increased frequencies of both chromosome abnormalities (excluding aneuploidy) and SCEs were found in the mothers of the three abnormal foetuses, compared with the remaining exposed animals, but the differences were not statistically significant ($P < 0.08$). No statistically significant differences were found between the two flocks when considering both chromosome abnormalities and SCEs.

Table III. Percentages of normal and abnormal cells in herds exposed to dioxins and control

Animals	Cells examined *n*	Normal cells		Abnormal cells	
		n	%	*n*	%
Exposed (24)	1257	600	47.7	657	52.3
Control (11)	633	453	71.7	179	28.3

Discussion

Chemical analyses

The high levels of dioxins found in the milk fail to be clearly explained by the analyses performed on the grass, soil and water where the two flocks were normally pastured. Indeed, all values found were below those permitted (Table I), although the two samples performed are certainly inadequate to screen the whole area of pasturage. European Community directive 2003/57/CE (European Commission, 2003) established a value of 0.75 pg WHO toxicity equivalent (TE)/g dioxins in feed with 12% water, however, no indication of the percentage of water was reported when analysing grass, although the analyses refer to grass dried at 80°C. This aspect is very important, because percentages of water >12% may affect the results of the analyses. Furthermore, it is difficult to establish how much feed (and dioxins) the two flocks ate given that they were only fed on pasture. This aspect should be further investigated, especially in cattle and river buffalo, where feed quantities are known.

Table IV. Percentages of chromosome abnormalities (aneuploidy, gaps, chromatid breaks, chromosome breaks and fragments) with respect to both abnormal cells (AC) and total cells examined (TC)

Animals	Abnormal cells														
	Aneuploidy (2n < 54)			Gaps			Chromatid breaks			Chromosome breaks			Fragments		
	n	AC (%)	TC (%)	*n*	AC (%)	TC (%)	*n*	AC (%)	TC (%)	*n*	AC (%)	TC (%)	*n*	AC (%)	TC (%)
Exposed (24)	195	29.7	15.5	821	125.0	65.3	100	15.2	8.0	30	4.6	2.4	12	1.8	1.0
Control (11)	97	54.2	15.3	75	41.9	11.8	16	8.9	2.5	5	2.8	0.8	1	0.6	0.2

Table V. Number of chromosome abnormalities (CA) (gaps, chromosome breaks, chromatid breaks and fragments) in diploid cells (2n = 54), aneuploid cells (2n < 54) and total cells of animals exposed to dioxins and controls

Animals (*n*)	Diploid cells			Aneuploid cells			Total cells		
	n	CA *n*	Mean/cell ± SD	*n*	CA *n*	Mean/cell ± SD	*n*	CA *n*	Mean/cell ± SD
Exposed (24)	1064	803	0.76 ± 1.15[a]	195	148	0.77 ± 1.11[a]	1257	963	0.77 ± 1.14[a]
Control (11)	536	97	0.18 ± 0.45	97	14	0.15 ± 0.44	633	112	0.18 ± 0.45

[a] $P < 0.001$.

Table VI. Animals, cells studied and SCE mean values in cells of exposed animals, abnormal foetuses and controls

Animals (*n*)	Cells examined (*n*)	SCE/cell	
		(*n*)	(mean ± SD)
24 (exposed)	600	5640	9.4 ± 3.7[a]
1 (abnormal foetus)	41	447	10.9 ± 4.4[a]
11 (control)	294	2086	7.1 ± 3.0

[a]$P < 0.001$.

Abortions and abnormal foetuses

The percentages of abortions were very high in the exposed flocks compared with the controls (Table II). This event may be correlated with the high percentage (52%) of animals affected by *Brucella abortis*. As regards the percentages of abnormal foetuses (2.4 and 2.5%), the breeders did not remember having seen such abnormalities during the last 50 years. This event could be related to the presence of dioxins, although more data are necessary to confirm this correlation. However, no abnormal foetuses were ever observed in the control group.

Cytogenetic analyses

The percentage of abnormal cells was higher (almost double) in the exposed flocks compared with that in the controls (Table III). However, no differences were noted between the percentages of aneuploid cells (all hypoploid) in exposed and control animals (Table IV), when considering total cells examined, although these percentages appeared very high in cells of both exposed and control animals. Only now are we beginning to monitor this biological aspect of cells of domestic cattle thanks to the use of digital cameras which allow all metaphases to be captured, enabling one to determine the exact number of chromosomes per metaphase. Since both controls and exposed had high frequencies of aneuploid cells which were exclusively hypoploid without any increase in hyperploid cells, the likelihood of the observed increase in aneuploid cells being due to technical artefacts (such as hypotonic treatment, fixation and chromosome spreading) cannot be ruled out. Percentages of abnormal cells were noticeably higher (from 2 to 4 times) in the exposed flocks, compared with those found in the controls, when considering gaps, chromatid breaks, chromosome breaks and fragments (Table IV). This is particularly evident when considering these abnormalities in diploid and aneuploid cells of exposed cells and controls (Table V). Indeed, the number of chromosome abnormalities was considerably higher (>4-fold) in both diploid and aneuploid cells of exposed animals, compared with those observed in the controls, these differences being highly significant ($P < 0.001$) (Table V).

Both chromosome abnormalities (excluding aneuploidy) and SCEs were significantly higher than observed in the controls (Tables V and VI) ($P < 0.001$), indicating enhanced chromosome fragility in the cells of flocks exposed to dioxins. The control SCE values are similar to our earlier investigations on two other breeds from our laboratory (Di Meo *et al*., 2000), further supporting the control value in the present study. The SCE test has previously been used in our laboratory to check the normal baseline level for other domestic species (reviewed in Di Meo *et al*., 2000) and to compare cattle with normal and abnormal (carriers of rob1;29) karyotypes (Rangel-Figueiredo *et al*., 1995).

Although cytogenetic tests performed in both humans and animals after *in vivo* and *in vitro* exposure to dioxins, in particular to TCDD, have given contradictory results (Tenchini *et al*., 1983; Kaye *et al*., 1985; Lim *et al*., 1987; Brook *et al*., 1988; Zober *et al*., 1993; Kamiguchi and Tateno, 2002), studies at the molecular level demonstrated that TCDD induces localized and discontinuous changes in chromatin structure (Okino and Whitlock, 1995). This could explain the increasing number of gaps, chromatid and chromosome breaks, fragments and SCEs found in the cells of exposed animals. Furthermore, TCDD is cytostatic to some cell types (Vogel and Abel, 1995; Wang *et al*., 1998; Elferink *et al*., 2001) but not others (reviewed by Santini *et al*., 2001). The basis for this phenomenon remains unclear.

The biological effects of TCDD are mediated by the aromatic hydrocarbon receptor (AHR), an intracellular protein and ligand-activated transcription factor that forms a complex with the aryl hydrocarbon nuclear translocator (ARNT) protein. This AHR–ARNT complex binds to TCDD and activates genes encoding for TCDD metabolizing enzymes, although AHR polymorphism seems to be the cause of the diverse responses of mammals (reviewed by Moriguchi *et al*., 2003). This may explain the different results obtained by various authors using different tests, including cytogenetic ones.

Recent studies in the area of Seveso revealed completely restored ecological conditions since both mutagenic and cytogenetic tests revealed no biological risks (Garagna *et al*., 1999, 2001). It is also important to remember that the US Environmental Protection Agency (EPA) has revised its earlier conclusions about TCDD by upgrading it from a 'probable' to a 'known' human carcinogen, TCDD cancer potency being 30 times higher than that estimated in 1985 by the EPA (Kaiser, 2000).

The preliminary results obtained suggest the advisability of extending our studies to larger samples of animals of different species and in different areas of Campania, especially those where high levels of dioxins have been found in the milk mass. Chemical analyses on human milk should also be performed to check for possible contamination with dioxins. These investigations should be accompanied by epidemiological studies in both humans and livestock to check the frequencies of abnormal foetuses, reproductive disorders and cancers (humans).

References

Bertazzi,P.A., Consonni,D., Bacchetti,S., Rubagotti,M., Maccarelli,A., Zocchetti,C. and Pesatori,A.C. (2001) Health effects of dioxin exposure: a 20 year mortality study. *Am. J. Epidemiol*., **153**, 1031–1034.

Brook,A.L., Jordan,S.W., Bose,J., Smith,J. and Allison,D.C. (1988) The cytogenetic and hepatotoxic effects of dioxin on mouse liver cells. *Cell Biol. Toxicol*., **4**, 31–40.

Chapman,D.E. and Schiller,C.M. (1985) Dose-related effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in C57BL/6J and DBA/2 J mice. *Toxicol. Appl. Pharmacol*., **78**, 147–157.

Di Meo,G.P., Perucatti,A., Fornataro,D., Incarnato,D., Ferrara,L., Matassino,D. and Iannuzzi,L. (2000) Sister chromatid exchange in chromosomes of sheep (*Ovis aries*). *Cytobios*, **101**, 71–78.

Elferink,C.J., Nie-Lin,G. and Levine,A. (2001) Maximal aryl hydrocarbon receptor activity depends on an interation with the retinoblastoma protein. *Mol. Pharmacol*., **59**, 1–10.

European Commission (2003) European Community directive 2003/57/CE. *Official Gazette*, L Serie, no. 151, 38–41.

Garagna,S., Rubini,P.G. and Redi,C.A. (1999) Recovering Seveso. *Science*, **283**, 1268–1269.

Garagna,S., Zuccotti,M., Vecchi,M.L., Rubini,P.G., Capanna,E. and Redi,C.A. (2001) Human-dominated ecosystems and restoration ecology: Seveso today. *Chemosphere*, **43**, 577–585.

Jorling,T. (2000) No longer a source of dioxin. *Science*, **290**, 934–935.
Kaiser,J. (2000) Just how bad is dioxin? *Science*, **288**, 1941–1944.
Kamiguchi,Y. and Tateno,H. (2002) Radiation- and chemical-induced structural chromosome aberrations in human spermatozoa. *Mutat. Res*., **504**, 183–191.
Kaye,C.I., Rao,S., Simpson,S.J., Rosenthal,F.S. and Cohen,M.M. (1985) Evaluation of chromosome damage in males exposed to agent orange and their families. *J. Craniofac. Genet. Dev. Biol*., **1** (suppl.), 259–265.
Kociba,R.J. (1991) Rodent bioassays for assessing chronic toxicity and carcinogenic potential of TCDD. In Gallo,M.A., Sheuplein,R.J. and van der Heijden,K.A. (eds), *Biological Basis Risk Assessment of Dioxins and Related Compounds*. Cold Spring Harbor Laboratory Press, Plainview, NY, pp. 3–12.
Lim,M., Jacobson-Kram,D., Bowman,R.E. and Williams,J.R. (1987) Effect of chronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on sister chromatid exchange levels in peripheral lymphocytes of the rhesus monkey. *Cell Biol. Toxicol*., **3**, 279–284.
Manz,A., Berger,J., Dwyer,J.H., Flesch-Janys,D., Nagel,S. and Waltsgott,H. (1991) Cancer mortality among workers in chemical plant contaminated with dioxin. *Lancet*, **338**, 959–964.
McGregor,D.B., Partensky,C., Wilbourn,J. and Rice,J.M. (1998) An IARC evaluationof polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans as risk factors in human carcinogenesis. *Environ. Health Perspect*., **106** (suppl. 2), 755–760.
Moriguchi,T., Motohashi,H., Hosoya,T. *et al*. (2003) Distinct response to dioxin in an arylhydrocarbon receptor (AHR)-humanized mouse. *Proc. Natl Acad. Sci. USA*, **100**, 5652–5657.
Okino,S. and Whitlock,J.P. (1995) Dioxin indices localized, graded changes in chromatin structure: implications for *Cyp1A1* gene transcription. *Mol. Cell. Biol*., **15**, 3714–3721.
Pesatori,A.C., Consonni,D., Bachetti,S., Zocchetti,C., Ronzini,M., Maccarelli,A. and Bertazzi,P.A. (2003) Short- and long-term morbidity and mortality in the population exposed to dioxin after the ''Seveso accident''. *Ind. Health*, **41**, 127–138.
Pirkle,J.L., Wolfe,W.H., Patterson,D.G., Needham,L.L., Michalek,J.E., Miner,J.C., Peterson,M.R. and Phillips,D.L. (1989) Estimates of the half-life of 2,3,7,8-tetrachlorodibenzo-p-dioxin in Vietnam veterans of Operation Ranch Hand. *J. Toxicol. Environ. Health*, **27**, 165–171.
Rangel-Figueiredo,M.T., Di Meo,G.P. and Iannuzzi,L. (1995) Sister chromatid exchange (SCE) in cattle: a comparison between normal and rob (1;29)-carrying karyotypes. *Hereditas*, **123**, 25–29.
Rier,S. and Foster,W.G. (2002) Environmental dioxins and endometriosis. *Toxicol. Sci*., **70**, 161–170.
Santini,R.P., Myrand,S., Elferink,C. and Reinars,J.J. (2001) Regulation of Cyp1a1 induction by dioxin as a function of cell cycle phase. *J. Pharmacol. Exp. Ther*., **299**, 718–728.
Tenchini,M.L., Crimaudo,C., Paccetti,G., Mottura,A., Agosti,S. and De Carli,L. (1983) A comparative cytogenetic study on cases of induced abortion in TCDD-exposed and nonexposed women. *Environ. Mutagen*., **5**, 73–85.
Vogel,C. and Abel,J. (1995) Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on growth factor expression I the human breast cancer cell line MCF-7. *Arch. Toxicol*., **69**, 259–265.
Wang,W., Smith,R. and Safe,S. (1998) Aryl hydrocarbon receptor-mediated antiestrogenicity in MCF-7 cells: modulation of hormone-induced cell cycle enzymes. *Arch. Biochem. Biophys*., **356**, 239–248.
Wolfe,W.H., Michalek,J.E., Miner,J.C., Pirkle,J.L., Caudill,S.P., Patterson,D.G. and Needham,L.L. (1994) Determinants of TCDD half-life in veterans of operation ranch hand. *J. Toxicol. Environ. Health*, **41**, 481–488.
Zober,A., Ott,M.G., Fleig,I. and Heidemann,A. (1993) Cytogenetic studies in lymphocytes of workers exposed to 2,3,7,8-TCDD. *Int. Arch. Occup. Environ. Health*, **65**, 157–161.

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