International Symposium on Environmental Endocrine Disrupters 2000

Saturday, December 16 - Monday, December 18, 2000

セッション2

2000年12月17日（日）

Session 2

Sunday, December 17, 2000

健康影響

Potential Effects on Human Health

Epidemiologic Evidence for Endocirine Disruption: Studies in North Carolina, U.S.A. and Mexico

Walter J. Rogan
National Institute of Environmental Health Sciences (NIEHS)

DDT was banned, in part, because it interfered with estrogen metabolism in pelagic birds, resulting in unhatchable eggs. Many persistent environmental chemicals can occupy estrogen receptor sites; in addition, certain PCBs occupy thyroid hormone receptor sites, and DDE can block the androgen receptor. The laboratory evidence is sound; however, few data exist on whether such activity is actually occurring in human beings. Two phenomena relevant to children that are plausibly the result of environmental hormones have been reported. The length of time that a woman lactates is, in part, a function of the low estrogen levels that occur post partum. Two studies (*Rogan WJ, Gladen BC, McKinney JD, Carreras N, Hardy P, Thullen JD, Tingelstad J, and Tully M. PCBs and DDE in human milk: effects on growth, morbidity, and duration of lactation. Am J Public Health 77: 1294-1297, 1987 and Gladen BC, Rogan WJ. DDE and shortened lactation in a northern Mexican town. Am J Pub Health, 85:504-8, 1995*) have shown that women with higher levels of DDE, a weak estrogen, wean earlier. In Taiwan, children whose mothers were poisoned by PCBs and polychlorinated dibenzofurans have ectodermal defects and developmental delay. As the boys go through puberty, their stages of development are normal but their penises are small. Both PCBs and DDE can affect the onset of puberty in laboratory animals. We have followed 600 North Carolina children from birth through puberty. Girls with higher perinatal exposures to PCBs experience the onset of the earlier stages of puberty earlier, but the effect is not consistent through puberty and not statistically significant. Boys with higher DDE exposures and girls with higher PCB exposures are heavier at age 14 years; the effect is large (several kilos at the extreme) robust and statistically significant (*Gladen BC, Ragan NB, Rogan WJ. Pubertal growth and development and prenatal and lactational exposure to polychlorinated biphenyls and dichlordiphenyl dichloroethene. J Pediatr 136: 490-496, 2000*). Although there is no direct evidence linking secular trends in endocrine illnesses to environmental chemicals, there are now several studies with individual measurements of exposure and plausible outcomes showing alteration of hormonally controlled processes from relatively low exposures.

Environmental Exposure to Polychlorinated Biphenyls (PCBs) and Dioxins. Consequences for Lactation Performances and Long Term Brain Development of the Child. A Review of the Longitudinal Dutch PCB/dioxin Study[1 2 3 4]

Ernst Rudolf Boersma
University Hospital Groningen

Polychlorinated biphenyl's (PCBs) and dioxins are lipophilic, chemically stable, and highly resistant to biological detoxification. Worldwide, contaminated food from animal origin is the major source (>90%) of human exposure. Continuous consumption of small amounts in food, in combination with a low rate of metabolic degradation, will lead to high body levels at reproductive age. Given the experimental evidence that PCBs exert an estrogenic effect, and estrogens are known to suppress human lactation, we investigated the effect of maternal PCB body burden on 24 hrs lactation performances.

From early gestation relatively large amounts of PCBs are found in fat of all fetal body compartments, including subcutaneous tissue, brain, and liver. Also after birth large amounts of PCBs and dioxins are transferred to the child through breast-feeding. On the contrary, formula milks are free of these substances. Given these circumstances and considering their potential developmental neurotoxicity, we investigated the long-term effects of prenatal and postnatal exposure to PCBs and dioxins on brain development in a breast-fed and a formula fed group of infants.

Methods. A total of 418 mother/infant pairs were included. Half of the mothers gave exclusively breast feeding (BF) for at least 6 weeks. In a subgroup of 102 mothers 24-hrs breast milk volume and milk fat content was measured at 2 and at 6 weeks after delivery. After birth 90-95% of all infants were examined at 2 weeks, 18 months, 3.5 years and at 6 years of age. As a measure of prenatal exposure PCBs were analyzed in cord and maternal blood. Postnatal exposure was reflected by PCB and dioxin levels measured in breast and formula milk. To estimate the difference in degree of exposure between breast and formula fed infants plasma PCB levels were determined in both feeding groups at 42 months of age. Neurological and cognitive development were taken as outcome variables at 18, 42 months and at 6 years of age. At 18 and at 42 months of age, neurological condition was evaluated according to the age-specific examination described by Hempel and Touwen, whereas at 6 years of age by means of the method according to Touwen. Separately, as an indicator for the integrity of brain function, fluency of movements was scored. Cognitive abilities were measured at 18 months by means of the Bayley Scales of Infant Development, at 42 months of age by the Kaufman Assessment Battery for Children (K-ABC) and at 6 years of age by the McCarthy Scales. Multivariate regression models were applied to analyze associations of measured exposure variables and a wide range of confounding perinatal and maternal variables with the outcome measurements.

Results. After adjustment for confounding factors maternal PCB body burden was inversely related to 24-h breast milk volume and milk fat content. Follow up of the infants revealed at *18 months of age* that cognitive development was not affected by either pre- or postnatal exposure to the measured PCBs and dioxins. However, neurological examination showed an adverse effect of prenatal exposure to the measured pollutants on the neurological optimality score. Also at *42 months of age* we found negative associations between prenatal PCB exposure and cognitive development. However, postnatal exposure to the measured pollutants did not affect cognitive development. Neurological development was not associated by either pre- or postnatal exposure to PCBs and dioxins. At *6 years of age* the preliminary results revealed no evidence that cognitive and neurological development were affected by

either pre- or postnatal exposure to these pollutants.
Despite 4 times higher degree of PCB exposure levels at 42 months of age in breast fed children versus their formula fed counterparts, we found at all follow up examinations (at 18, 42 months and at 6 years of age, respectively) a beneficial effect of breast feeding on the quality of movements, in terms of fluency.
Conclusion. In Western Europe human breast milk volume and fat content is adversely affected by presently encountered background concentrations of PCBs. This may point to an estrogenic activity of the PCB mixture to which the Dutch women have been exposed during their life. Long term follow up of the children since birth showed, similar to studies in the USA, an adverse effect of transplacental exposure to PCBs on brain development. Despite substantial higher PCB exposure levels in BF children as compared to their formula fed counterparts a beneficial effect of breast-feeding on the fluency of movement at 18, at 42 as well as at 72 months of age was found after adjustments for social, obstetric, and perinatal differences. Follow up of the study population may shed light on the possible endocrine disrupting effects of these environmental pollutants on hormone-related pathology later in life.

References:
1. Huisman M (1996) Effects of early infant nutrition and perinatal exposure to PCBs and dioxins on neurological development. A study of breast-fed and formula-fed infants [Dissertation]. Groningen. University of Groningen. ISBN 90-3670688-2.
2. Koopman-Esseboom C (1995) Effects of perinatal exposure to PCBs and dioxins on early human development [Dissertation].Rotterdam. University of Rotterdam. ISBN 90-75340-03-6.
3. Lanting CI (1999) Effects of perinatal PCB and dioxin exposure and early feeding on child development [Dissertation]. Groningen. University of Groningen. ISBN 90-3671002-2
4. Patandin S (1999) Effects of environmental exposure to Polychlorinatedbiphenyls and dioxins on growth and development in young children. [Dissertation].Rotterdam. University of Rotterdam. ISBN 90-9012306-7.

Acknowledgements The Dutch PCB/dioxin study was initiated by the Dutch Government and is currently part of an international research project sponsored by the European Community (contract no. EV5V-CT92-0207). Collaborating Centers of the Dutch PCB dioxin study are: Div of Neonatology, Sophia Children Hospital, Erasmus University Rotterdam [project leaders Prof PPJ Sauer (till 1998) /Dr N Weisglas-Kuperus] and the Perinatal Nutrition & Development Unit/Dept. of Developmental Neurology, University Hospital Groningen [project leaders Prof ER Boersma/Prof BCL Touwen], the Netherlands. We thank all parents and their children for participating in this study.

Thyroid Status, Frequency of Selected Autoantibodies and Biomarkers in the Population Exposed to PCB and Other Organochlorines

Pavel Langer[1], Mária Tajtáková[4], Anton Kočan[2], Ján Petrík[2], Ján Kaušitz[3], Jana Chovancová[2], Beáta Drobná[2], Stanislav Jursa[2], Marián Pavúk[2], Tomáš Trnovec[2], Hans-Joachim Guretzki[5], Elena Šeböková[1], Iwar Klimeš[1]

[1]Slovak Academy of Sciences, [2]Slovakia Institute of Preventive and Clinical Medicine, Slovakia
[3]National Institute of Oncology, Slovakia; [4]1st Clinic of Internal Medicine, Slovakia;
[5]Brahms Diagnostica, Germany

Introduction. During last several decades the global environment has been polluted by several organochlorinated substances. Since not only their structure closely resembles that of thyroid hormones or nonsteroid estrogens, but namely they are disrupting estrogen, androgen and thyroid hormone systems in the broadest sense, they have been defined as "endocrine disruptors" which "interfere with the production, release, transport, metabolism, binding, action or elimination of natural hormones" (Kavlock et al. 1996).

Objective. Certain factory produced about 22,000 tons of PCB between 1955-85. There was no protecion of workers and the waste was dumped directly to the river without any protective treatment which resulted in heavy contamination of underground and superficial waters, sediments, soil and food chain. Although the production was terminated in 1985, high levels of total PCB in environmental and human samples were still found in 1994 and 1998 which means that the population is living in highly contaminated area for more than four decades. We intended to screen selected health effects of such unique pollution which, however, was possible only after the change of political regime in 1989/90, since before that any information about the pollution has been strictly banned.

Methods. We examined 238 factory employees (EMPL) and 572 controls (CON) in the first survey (1994) and 101 contaminated subjects (including EMPL) and 360 CON in the second one (1998). Thyroid volume (ThV) and structure were examined by ultrasound. Thyrotropin (TSH), anti-thyroperoxidase (anti-TPO), anti-GAD (Glutamic Acid Decarboxylase) and anti-IA2 in serum were measured (1994 and 1998). In 1994 also total thyroxine (TT4), anti-thyroglobulin (anti-TG) and anti-TSHreceptor (anti-TSHr) antibodies as well as beta2-microglobulin (beta2-MG), alpha-fetoprotein (AFP) and thymidine kinase (TK) were measured in serum. In 1998 organochlorines (PCB, hexachlorbenzene-HCB, hexachlorcyclohexane-HCH, DDE and DDT) were measured in serum, soil, waters and foods by congener specific analysis (microcapillary gas chromatography/mass spectrometry), while in 1994 only few data about that were available. Urinary iodine was measured in 808 subjects.

Results. In 1994 ThV was higher ($P<0.001$) in EMPL than in CON (mean ± SE: 18.85 ± 0.69 vs. 13.47 ± 0.48 ml; upper quartile: 22.9 vs. 15.3 ml) and in 1998 ThV was found to be related to organochlorine levels. In 1994, the frequency of anti-TPO ($P<0.05$), anti-TG ($P<0.05$) and anti-TSHr ($P<0.001$) was higher in EMPL than in CON and in 1998 that of anti-TPO was found to be related to organochlorine levels. In spite of those differences in ThV and antibodies, no considerable differences in TT4 and TSH were found.

Considering immunomodulatory changes shown by high frequency of thyroid antibodies, we retrospectively estimated the frequency of anti-GAD and anti-IA2. In 238 EMPL 41.6 % of anti-GAD positive were found vs. 10.3 % in 704 CON ($P<0.001$), while no differences were found in anti-IA2.

The frequency of low beta2-MG levels (<1.6 μg/ml) was 77 % in 242 EMPL vs. 23 % in 636 CON ($P<0.001$), while no changes were found in AFP and TK. One of possible explanations may be the impairment of

renal tubules resulting in increased urinary looses of beta2-MG.

In 1998, the levels of PCB (mean ± SE) were higher in 101 exposed subjects than in 360 CON (7300 ± 871 vs. 2045 ± 147 ng/g lipids; P<0.001), the respective levels of HCB being 1890 ± 156 vs. 1738 ± 95 (NS) and of DDE 3834 ± 279 vs. 3164 ± 137 (P<0.02), while these of HCH and DDT were negligibly low. After all 461 subjects were sorted according to PCB level and divided into deciles, the ThV in 1st-8th decile (N=368) was 13.8 ± 0.3 vs. 17.1 ± 0.9 ml in 9th+10th decile (N=93). The respective frequency of thyroid hypoechogenicity by ultrasound was 99/368 vs. 41/93 (P<0.02), of positive anti-TPO was 43/368 vs. 20/93 (P<0.02), of decreased TSH level (>0.15 mU/l) was 12/368 vs. 12/93 (P<0.001) and of anti-GAD was 62/368 vs. 28/93 (P<0.02).

Discussion and general conclusions. Since mandatory and well controlled consumption of iodized salt in Slovakia exists from early fifties and the satisfactory life-long iodine intake in the population was repeatedly confirmed, the observed thyroid changes could not result from local iodine deficiency in polluted area. Since the levels of PCB in polluted area were 3-4 times higher than in CON, PCB apparently played a leading role among the variety of organochlorines, although the second survey showed that even HCB and DDE might participate in the harmful actions.

The mechanism of harmful action of organochlorines on the thyroid still remains to be explained. According to our opinion (Langer et al.: Eur.J.Endocrinol. 139:402-409,1998), such changes presumably resulted from long-term and oscillating multiple effects on the thyroid hormone metabolism, TSH production and intrathyroid events including possible deterioration of cell membranes by lipophilic organochlorines facilitating the cross-talk between cellular antigens and modulated immune system resulting in autoimmune impairment of the thyroid tissue, and possible estrogen like effects on the thyroid etc. We are convinced that we are facing a new and intriguing steady state of the pituitary-thyroid system which might not be manifested by the persisting detectable abnormalities in hormone levels. Such steady state developed after such long-term exposure and we can only find the outlasting and perhaps more or less irreversible remnants or sequels of previous long-term development such as increased thyroid volume showing frequent hypoechogenicity and nodules and also increased prevalence of autoantibodies.

Evidence of Endocrine Disruption in Yucheng People Exposed Polychlorinated Biphenyls and Dibenzodioxins

Yue-Liang Leon Guo[1], Chen-Chin Hsu[2], Mei-Lin Yu[1]

[1]National Cheng Kung University Medical College

[2]Tainan Municipal Hospital

Introduction

The human health effects of PCBs, PCDFs, and dioxin are important to understand since these polyhalogenated aromatic hydrocarbons (PAHs) have been found in the serum of all populations ever studied and the many of the PAH congeners have long half lives in the human measured in years. Many human health studies have been conducted in cohorts uniquely exposed to these PAHs. In this discussion the human health effects identified in several cohorts will be compared and contrasted in an attempt to better understand the scope and severity of the effects found from these exposures and discuss potential mechanisms of the congeners, toxicity and the observed altered susceptibility of the cohorts.

Methods and Materials

The initial cohort to be discussed will be the cohort from Taiwan referred to as the Yucheng ("oil-disease") cohort. The Yucheng cohort consisted of Chinese in Taiwan exposed to PCBs and their heat-degradation products from the ingestion of contaminated rice oil in 1979[1]. The exposure levels, mortality and morbidity, reproductive history in women, findings of children at birth and later development will be compared with findings from high accidental exposures, Yusho ("oil-disease") of Japan, 2,3,7,8-TCDD exposure in Seveso, Italy, as well as those from cohorts with background levels of exposure. Evidence related to endocrine effects will be specifically discussed.

Results and Discussion

Exposed Yucheng people had initial serum levels estimated to be higher than 20,000 ppb lipid base for PCBs and 40,000 ppt lipid base for PCDFs. Fourteen years after the exposure, the Yucheng female adults had serum levels of PCDF up to two hundred times as high as found in the unexposed, closely matched controls, and serum levels of PCBs up to more than ten times the control subjects[2]. Lactation by breast-feeding reduced the serum levels in the Yucheng mothers, but increased serum levels in their children[3]. This level of exposure was similar to the level in Japan Yusho cohort[4], except that the levels of toxicants in the contaminated oil were lower in Taiwan, and the duration of exposure was much longer. The median exposure level in cohort from Seveso was lower than the two Asia episodes in terms of dioxin toxic equivalencies[5].

The Yucheng cohort exposed as adults was found to have increased mortality secondary to chronic liver diseases and cirrhosis, but similar rate of liver cancer as compared to the national death using standardized mortality ratio 12 year after the exposure[6]. These findings were different from Yusho and Seveso cohorts. A telephone health survey was conducted 14 years after the exposure[7], which showed that Yucheng men reported increased prevalence of having skin allergies, chloracne, headache, spine and joint diseases, and goiter. Yucheng women reported increased prevalence of having skin allergies, chloracne, headache, anemia, and goiter. These findings were to some extent similar to those from other exposed populations. However, different findings were also noted, which could be caused by difference in exposure levels, routes, and duration of follow-up.

Reproductive history of the women was compared with unexposed controls in a retrospective study on

Yucheng cohort[8]. Among Yucheng women, 4.2% reported a stillbirth since 1979, as compared with 1.7% in unexposed controls (P=0.068). More Yucheng women reported that one of their offspring had died during childhood (10.2% vs. 6.1%, P<0.05). Similarly, Hsu et al. reported that of the 39 Yucheng babies in utero during the time the mothers ingested the contaminated oil, 8 died in the first few years of life, mostly from perinatal conditions and respiratory infections[1].

Children of Yucheng women were born growth retarded, with dysmorphic physical findings, and delayed cognitive development as compared with unexposed children. The dysmorphic features included cola-colored hyperpigmented skin, hyperpigmented oral mucosa, chloracne, Meibomian gland swelling, natal teeth and fragile teeth, and deformed and pigmented nails[9]. Children born to the cohort after the initial exposure have been followed from birth to the present day. From 4 to 11 years of age, Yucheng children had reduced intelligence quotients compared to the unexposed controls[10]. Those children born immediately after maternal exposure were similarly affected in neurocognitive developments as those born 6 years after the exposure. In their age 8-14, Yucheng children had increased nail deformities[11]. The main nail findings were transverse coarse grooves and irregularly concaved depression in approximately one quarter of Yucheng children, with predilection for thumbs, followed by big toes and other fingers. Those children born closer to the mothers' intoxication had more nail deformities than those born later. The Yucheng children not only had increased respiratory infections reported by parents[12], but also increased chronic otitis media compared to the matched controls by examination in 1993[13].

In regards to endocrine disruption in the transplacentally and lactationally exposed subjects, Yucheng adolescent males had decreased capacity to understand spatial relationships as compared to their long-term closely matched males control subjects[14]. Possibility of loss in male advantage in visuospatial capability was suspected. No differences were identified when the exposed and none exposed females were compared. In prenatally exposed young men who had reached sexual maturity, sperm analysis showed increased abnormal morphology, reduced motility, and reduced capability of penetrating hamster oocytes[15].

Long-term follow-up of Yucheng cohort, when compared with other poisoning episodes, has been able to provide valuable information concerning the health effects of PCBs/PCDFs/PCDDs. Information about the toxicities, health effects, and dose-response relationship in directly exposed and perinatally exposed humans can be achieved. It has also been demonstrated that exposure to these chemicals caused prominent health effects in several outcome measures. People exposed perinatally are found to be one of the most susceptible group of humans to the toxic effects of these persistent organic pollutants.

Acknowledgment

This study is supported partially by the research grant #NSC88-2314-B-006-117 from the National Science Council, Taiwan, R.O.C.

Reference

1. Hsu, S.T., Ma, C.I., Hsu S.K.H., Wu, S.S., Hsu, N.H.M., Yeh, C.C., Wu, S.B. (1985) Environ Health Perspect. 59, 5.
2. Guo, Y.L., Ryan, J.J., Lau, B.P.Y., Yu, M.L., and Hsu, C.C. (1997) Arch Environ Contamin Toxicol. 33, 104.
3. Ryan, J.J., Hsu, C.C., Boyle, M.J., and Guo, Y.L. (1994) Chemosphere 29, 1263.
4. Masuda, Y., Schecter, A., and Rapke, O. (1998) Chemosphere 37, 1773.
5. Needham, L.L., Gerthoux, P.M., Patterson, D.G. Jr., Brambilla, P., Smith, S.J., Sampson, E.J., and Mocarelli, P. (1999) Environ Res. 80, S200.

6. Yu, M.L., Guo, Y.L., Hsu, C.C., and Rogan, W.J. (1997) Am J Indust Med. 31, 172.
7. Guo, Y.L., Yu, M.L., Hsu, C.C., and Rogan, W.J. (1999) Environ Health Perspect. 107, 715.
8. Yu, M.L., Guo, Y.L., Hsu, C.C., and Rogan, W.J. (2000) Int J Epidemiol. 29, in press.
9. Rogan, W.J., Gladen, B.C., Hung, K.L., Koong, S.L., Shih, L.Y., Taylor, J.S., Wu, Y.C., Yang, D., Ragan, N.B., and Hsu, C.C. (1988) Science 241, 334.
10. Chen, Y.C., Guo, Y.L., Hsu, C.C., and Rogan, W.J. (1992) JAMA 268, 3213.
11. Hsu, M. M.-L., Mak, C.-P., and Hsu, C. C. (1995) Br J Dermatol. 132, 427.
12. Ju, S.-H., Chen, Y.-J., Chen, Y.-C., and Hsu, C.-C. (1992) Pediatr. Res. 28, 93A.
13. Chao, W.Y., Hsu, C.C., and Guo, Y.L. (1997) Arch Environ Health 52, 257.
14. Guo, Y.L., Lai, T.J., Chen, S.J., and Hsu, C.C. (1995) B Environ Contamin Toxicol. 55, 8.
15. Guo, Y.L., Hsu, P.C., Hsu, C.C., and Lambert, G.H. (2000) Lancet, in press.

Dioxin and Human Health: 20 Years of Data from "Seveso", Italy

Paolo Mocarelli
University of Milano-Bicocca

The level of toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD or dioxin) in humans is still being debated (1). Data are provided by occupational, military and environmental exposure in adult males, with indirect assessment of TCDD original concentration (2, 3). Except for cases of chloracne, symptoms seem to have been transitory. A longitudinal health monitoring plan between 1976 and 1998 of the population of Seveso (about 17,000) affected by the fallout of a toxic cloud containing TCDD (4-6), released over that town and nearby areas by an explosion in a TCP (2,4,5-trichlorophenol)-producing factory on July 10, 1976 has been performed. It included monitoring of dermatological, obstetric, pediatric and neurological examination and biochemical, hematological, and immunological studies. Principal results after eight years from the accident indicate that chloracne was the only clinical alteration positively correlated to TCDD contamination levels even if it is not a sensitive sign of exposure.

Laboratory results showed minimal differences between exposed and controls in the period of acute exposure (1976-1977) for aspartate-aminotransferase, aline-aminotransferase, alkaline phosphatase, total proteins, complement hemolytic activity in serum, and for white blood cells, lymphocytes, and hemoglobin; after 1977, for gamma-glutamyltranspeptidase only. These differences were subclinical, faded and disappeared with time (7).

After 1998 we have been reviewing some of these outcomes on about 1500 people with TCDD serum level measurements out of 30000 blood samples drawn in 1976-79, kept frozen since then and measured after 1988 at Centers for Disease Control and Prevention (CDC), Atlanta, USA (8,9). We have been able to relate the original (1976) level with investigated outcomes. Here we summarize some results.

TCDD and immune system

The aim of this study was to verify if TCDD induced reversible and/or persistent alterations of the developing human immune system in children exposed after Seveso accident.

We considered about 400 subjects with TCDD serum measured on 1976-77 serum samples and evaluated some immunological parameters as total lymphocytes and, on a subset of 40 people, T and B lymphocytes, lymphocytes proliferation after mitogen (phytohemoagglutinin (PHA) and pkeweed mitogen (PWM) stimulation, immunoglobulins and complement activity (CH50). We compared these analytes in 1976-77 (10) and in 1992-98, after about 20 years from exposure. Evaluation of preliminary results shows some low effects related to TCDD dose in 1976.

Microsomal induction

TCDD is known to induce hepatic microsomal enzymes both in animals and in humans. Some studies have shown an incresed urinary excretion of D-glucaric acid after hepatic microsomal enzymes induction. In 1976 to 1981 we measured urinary D-glucaric acid content in urine samples to verify whether among Seveso people there was an incresed hepatic enzymes activity (11).

Children with chloracne showed significantly higher levels of D-glucaric acid excretion than children without chloracne living in the same zone. Up to 3 years after the exposure both adult and children showed a statistically

significant increased D-glucaric acid excretion, compared to the control groups.

Considering TCDD-serum concentration in 1976-77, the increase of D-glucaric acid excretion is observed in people with TCDD serum levels higher than 1000 ppt.

Sex ratio at birth

A striking skewing of sex ratio at birth (males/males+females) with excess of female ($p<0.001$) from parents highly exposed to TCDD has been described for the period 1977-84 (12). Very recently (13) this effect has been shown to be permanently related only to father exposure with the pre and puberty period being a very sensitive period and it has been recently confirmed (14) in a dioxin induced chloracneic Austrian cohort.

The TCDD concentrations by which this lower sex ratio is induced in Seveso group are only about 20 times the estimated average concentration corrently found in human beings in industrialized countries. The human male reproductive system is demonstrated for the first time to be very sensitive to dioxin. This fact can have important public-health implications due to the very different individual sensitivity in humans.

References

1. Grassman JA, Masten SA, Walker NJ and Lucier GW; *Environ. Health Persp*. **1998**, 106, 761.
2. Wolfe WH, Michalek JE, Miner JC, Rahe A, Silva J, Thomas WF, Grubbs WD, Lustik MB, Karrison TG, Roegner RH and Williams DE; JAMA **1990**, 264, 1824.
3. Svensson BG, Nilson A, Hansson M, Rappe C, Akesson B and Skerfving S; *N. Engl. J. Med.* 1991, 324, 8.
4. Di Domenico A, Cerlesi S and Ratti S; *Chemosphere* **1990**, 20, 1559.
5. Mocarelli P, Marocchi A, Brambilla P, Gerthoux P, Beretta C, Colombo L, Bertona M, Sarto C, Tramacere P, Mondonico A, Crespi C, Signorini S and Brivio R; *Toxic. Subst. J.* **1992**, 12, 151.
6. Bertazzi PA, Bernucci I, Brambilla G, Consonni D and Pesatori A; *Environ. Health Persp*. **1998**, 106, 625.
7. Mocarelli P, Marocchi A, Brambilla P, Gerthoux PM, Colombo L, Mondonico A, Meazza L. p 95-107, in *Biological Basis for risk assessment of Dioxins and Related Compounds. Banbury Report* 35, Ed. M.A. Gallo, R.J. Scheuplein and K.A. Van Der Heijden, New York: *Cold Spring Harbor Laboratory Press*, **1991**.
8. Patterson DG, Hampton L, Lapeza CR, Belser WT, Green V, Alexander L and Needham LL; *Anal. Chem*. **1987**, 59, 2000.
9. Needham LL, Gerhtoux PM, Patterson DG Jr, Brambilla P, Turner WE, Beretta C, Pirkle JL, Colombo L, Sampson EJ, Tramacere PL, Signorni S, Meazza L, Carreri V, Jackson RJ and Mocarelli P; *Teratogenesis Carcinogenesis Mutagenesis* **1997/98**, 17, 225.
10. Sirchia GG and the Group for Immunological Monitoring: Exposure to TCDD. p. 234-266, in *Plans for Clinical and Epidemiological Follow-up after Area-Wide Chemical Contamination: Proceedings of an International Workshop*, Ed. L. Dardanoni and R.W. Miller, Washington, DC: National Academy Press, **1982**.
11. Ideo G, Bellati G, Bellobuono A, Mocarelli P, Marocchi A and Brambilla P; *Clin. Chim. Acta* **1982**, 120, 273.
12. Mocarelli P, Brambilla P, Gerthoux PM, Patterson DG Jr and Needham LL; *Lancet* **1996**, 348, 409.
13. Mocarelli P, Gerthoux PM, Ferrari E, Patterson DG jr, Kieszak SM, Brambilla P, Vincoli N, Signorini S, Tramacere P, Carreri V, Sampson EJ, Turner WE, Needham LL; *Lancet* **2000**, 355, 1858-1863.
14. Moshammer H, Neubergher M; *Lancet* **2000**, 356, 1271-1272.