



Aryl-hydrocarbon receptor-dependent pathway and toxic effects of TCDD in humans: a population-based study in Seveso, Italy

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Abstract

Approximately 20 years after the Seveso, Italy accident, we conducted a population-based study to evaluate the impact of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exposure upon immune and mechanistically based biomarkers of dioxin response in humans. TCDD toxic effects are known to be mediated by the aryl-hydrocarbon receptor (AhR). We randomly selected 62 study subjects from the highest exposed zones and 59 from the surrounding non-contaminated area. Current lipid-adjusted plasma TCDD concentrations in these subjects ranged from 3.5 to 90 ng/kg (or ppt) and were negatively associated with plasma IgG concentrations ($r = -0.35$; $P = 0.0002$). The expression of genes in the AhR-dependent pathway, including AhR, aryl-hydrocarbon receptor nuclear translocator (ARNT), CYP1A1, and CYP1B1 transcripts, and the CYP1A1-associated 7-ethoxyresorufin-*O*-deethylase (EROD) activity was measured in lymphocytes. AhR mRNA levels in uncultured lymphocytes were negatively associated with plasma TCDD ($P = 0.03$). When mitogen-induced lymphocytes were cultured with 10 nM TCDD, all AhR-dependent genes were induced 1.2- to 13-fold. In these cells, plasma TCDD was associated with decreased EROD activity. Markers within the AhR pathway were correlated with one another.

Our findings suggest the presence of long-term effects in the subjects exposed to TCDD after the Seveso accident.
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1. Introduction

Dioxins are widespread contaminants of the environment generated as by-products in the manufacturing of chlorophenols and chlorophenoxy herbicides, as well as in other common processes, such as fossil fuel and wood combustion, paper-pulp bleaching using free chlorine, metal processing, and waste incineration. Tetrachlorodibenzo-*p*-dioxin (TCDD), the most toxic congener, has a long biological half-life in humans (≥ 7 years) and is classified by the International Agency for Research on Cancer (IARC, 1997) as a human carcinogen, based on a plausible mechanism involving the highly conserved aryl-hydrocarbon receptor (AhR), animal models and human data from industrial exposures and accidents.

Genetic and biochemical studies indicate that the AhR is necessary for most of the toxic effects of TCDD, such as tumor promotion, thymic involution, craniofacial anomalies, skin disorders and alterations in the endocrine, immunological and reproductive systems. AhR is a nuclear receptor and transcription factor. In the presence of TCDD, AhR forms an active heterodimer with the aromatic hydrocarbon nuclear translocator (ARNT/HIF-1 β) and induces the transcription of xenobiotic metabolizing enzymes, such as cytochrome P4501A1 (CYP1A1) and P4501B1 (CYP1B1), as well as other genes (Whitlock, 1999). Prolonged expression of CYP1A1 may increase the likelihood of deleterious DNA lesions, due to an increase in the generation of genotoxic metabolites and reactive oxygen species. Similarly, CYP1B1 may be involved in the mechanism of carcinogenesis through its metabolism of 17 β -estradiol and bioactivation of polycyclic aromatic hydrocarbons and arylamines.

Both CYP1A1 and CYP1B1 mRNA levels, measured by quantitative RT-PCR in peripheral lymphocytes, have been proposed as biomarkers of TCDD biological effective dose in humans.

The immune system is one of the most sensitive targets of TCDD (Birnbaum and Tuomisto, 2000). TCDD inhibits immunoglobulin secretion and decreases resistance to bacterial, viral, and parasitic infections in exposed animals. In humans, increased incidence of and mortality from lymphatic tumors have been found in exposed subjects, even though there is a lack of consistency among studies (Bertazzi et al., 2001; IARC, 1997).

The Seveso, Italy, industrial accident exposed several thousand people to substantial quantities of TCDD. The accident took place in the summer of 1976, when the temperature and pressure surged in an improperly maintained reaction vessel in the trichlorophenol production department of a chemical plant near the town of Seveso, 25 km north of Milan; given the concomitant failure of a safety device, the contents of the reactor were vented directly into the atmosphere.

The level and extent of the environmental contamination were documented by dioxin soil measurements in a wide area along the direction of the prevailing winds. Three contamination zones were delimited: the most heavily contaminated, zone A, included nearly 800 residents; zone B (about 6000 subjects) was right after along the fallout path of the chemical cloud; and zone R (about 39,000) with low-level and patchy contamination, represented a gray, circular strip between the highly contaminated zones and the surrounding territory (Bertazzi et al., 2001). One of the earliest accident-related health effects was chloracne in children who were outdoors and in the path of the toxic cloud. In the following years, under the supervision of an international steering committee, other health outcomes possibly linked to TCDD exposure were investigated, including spontaneous abortions, cytogenetic abnormalities, congenital malformations, impaired liver function and lipid metabolism, and immunologic and neurologic impairment. In 1984, the committee concluded its work and stated that the only ascertained effect of dioxin exposure was chloracne but that long-term studies were needed.

Epidemiology investigations have been conducted on a cohort including all subjects residing in any of the contaminated zones (A, B, or R) on the date of the accident or in the following 10 years, and a reference population (approximately 230,000 subjects) from the surrounding non-contaminated area (non-ABR zone). Mortality studies (Bertazzi et al., 2001) showed an increased risk of lymphatic/hemopoietic neoplasms in both genders, rectum and lung cancer in males, and diabetes and COPD in females (Table 1). Incidence studies confirmed the increased risk of lymphatic/hemopoietic cancer (Bertazzi et al., 1999).

Approximately 20 years after the exposure, we designed a population-based study to evaluate the impact of TCDD exposure upon immune and

Table 1

Observed and expected number of deaths, rate ratios (RRs), and 95% confidence intervals (95% CIs) for selected causes of death^a in the population exposed to dioxin (zones A and B) after the Seveso, Italy accident, 1976–1996

	Females			Males		
	Observed	Expected	RR ^b (95% CI)	Observed	Expected	RR ^b (95% CI)
All causes	307	308.5	1.0 (0.9–1.1)	438	436.2	1.0 (0.9–1.1)
All cancers	83	90.8	0.9 (0.7–1.1)	166	149.7	1.1 (1.0–1.3)
Lymphatic/hemopoietic	13	7.1	1.8 (1.1–3.2)	15	9.1	1.7 (1.0–2.8)
Lung	4	6.2	0.6 (0.2–1.7)	57	43.9	1.3 (1.0–1.7)
Rectum	3	2.7	1.1 (0.4–3.5)	10	3.8	2.4 (1.2–4.6)
Diabetes	20	11.6	1.7 (1.1–2.7)	6	7.7	0.8 (0.3–1.7)
Chronic ischemic heart disease	18	18.8	1.0 (0.6–1.5)	28	20.3	1.4 (0.9–2.0)
Chronic obstructive pulmonary disease	12	5.4	2.2 (1.2–4.0)	17	13.6	1.2 (0.8–2.0)

^a Complete results from the Seveso mortality study, 1976–1996, were reported in Bertazzi et al. (2001).

^b Adjusted for age and calendar period.

mechanistically based biomarkers of dioxin response in humans. We randomly selected 62 subjects from the most exposed zones (A and B) and 59 from the surrounding non-contaminated area (non-ABR) in order to obtain a study sample representative of the general population of the entire area. In individuals from zones A and B, elevated plasma TCDD levels (ranging from background values to 90 ng/kg lipid, or parts per trillion, ppt) were still present after a period of time approximately equivalent to two biological half-lives, with significantly higher levels in women (Landi et al., 1997). In contrast, other dioxin-like congeners were at background levels in both TCDD-exposed and non-exposed areas. TCDD levels in study subjects were within the range of body burdens associated with sensitive dioxin-dependent responses in animal studies, such as induction of CYP1A1 (DeVito et al., 1995).

2. Material and methods

The study subjects were recruited between December 1992 and March 1994. Informed consent was obtained from all participants and the local Institutional Review Board approved the study. The dioxin measurements in human plasma were performed at the CDC using a high resolution gas chromatography/high resolution mass spectrometry analysis (Patterson et al., 1987). Specifically, TCDD and 21 other dioxin or dioxin-like congeners were measured, including 10 dibenzofurans, four co-planar polychlorinated biphenyls and seven additional dibenzo-*p*-dioxins.

Results are reported in ppt, lipid adjusted. Of the 121 subjects, 11 samples (4 from zone B and 7 from zone non-ABR) were inadequate and were excluded from the analyses based on plasma TCDD. In another 23 subjects (9 from zone B and 14 from zone non-ABR), TCDD levels were determined to be below the detection threshold and so values were estimated by dividing the lipid-adjusted detection limit by $\sqrt{2}$.

We measured IgG, IgM, IgA, C3, and C4 concentrations in plasma using standard nephelometric methods.

We measured mRNA levels of the AhR, ARNT, CYP1A1, and CYP1B1 genes and 7-ethoxyresorufin-*O*-deethylase (EROD) activity in peripheral blood lymphocytes. Some AhR-dependent markers, such as CYP1A1 expression and EROD activity, are known to be only barely detectable in uncultured lymphocytes (Grassman et al., 1999; Masten et al., 1998). Therefore, in addition to the measurement of the AhR-related markers in uncultured lymphocytes, we also used cultured lymphocytes, stimulated with mitogen and treated with in vitro TCDD. Culture stimulation medium consisted of basal medium containing 1.25 μ g/ml phytohemagglutinin (Murex Diagnostics, Norcross, GA), 0.15% (v/v) pokeweed mitogen (Life Technologies) and 50 μ M 2-mercaptoethanol (Sigma). Half of the cells was treated with 10 nM TCDD (cells cultured with mitogen + 10 nM TCDD). The control cultured cells received an equal volume of stimulation medium without the TCDD (cells cultured with mitogen only). Both treated and untreated cells were cultured for 72 h. The levels of AhR, ARNT, CYP1A1, and CYP1B1 mRNA were measured using

quantitative competitive reverse transcription PCR (RT-PCR). Details on biological sample acquisition and storage, peripheral blood mononuclear cell culture, RNA isolation and quantitative RT-PCR primers and conditions, as well as on the EROD assay, were previously described (Landi et al., 2003).

Data on IgG, IgM, IgA, C3, and C4 levels between groups with different characteristics were analyzed using non-parametric univariate tests (Wilcoxon rank-sum, Kruskal–Wallis). Differences in gene expression and EROD activity between experiments performed in different cell culture conditions were evaluated using the paired *t*-test. Logarithmic transformations of measured mRNA levels and EROD activity were used to improve the fit to a normal distribution. Consequently, geometric means are reported. We used the unpaired Student's *t*-test to test for between-group differences of AhR-dependent markers.

We performed simple and multiple linear regression analyses to assess correlations between variables. Pearson's correlation coefficients (*r*) and Wald's test *t* values for simple and multiple regression analyses, respectively, are reported throughout the manuscript. Results on gene expression from uncultured cells with viability <75% (23 subjects) were excluded from the analyses. All *P*-values are two-sided. All analyses were performed with the use of the Stata statistical package Release 7.0 (Stata Corp., College Station, TX).

3. Results

3.1. Immunologic parameters

Subjects from the zones contaminated by TCDD showed lower plasma IgG than subjects from the surrounding reference (non-ABR) area (Fig. 1a). Median IgG levels were 1403 mg/dl in zone non-ABR (*n* = 58), 1294 mg/dl in zone B (*n* = 55; *P* = 0.03 versus non-ABR), and 1142 mg/dl in zone A, the most contaminated area (*n* = 7; *P* = 0.01 versus non-ABR). Plasma IgG progressively decreased with increasing lipid-adjusted TCDD plasma concentration (*r* = -0.35; *P* = 0.0002) (Fig. 1b) (Baccarelli et al., 2002). After adjusting for age, sex, smoking, and consumption of domestic livestock, the inverse association between plasma TCDD and IgG remained highly significant (*P* = 0.0004). A number of fac-

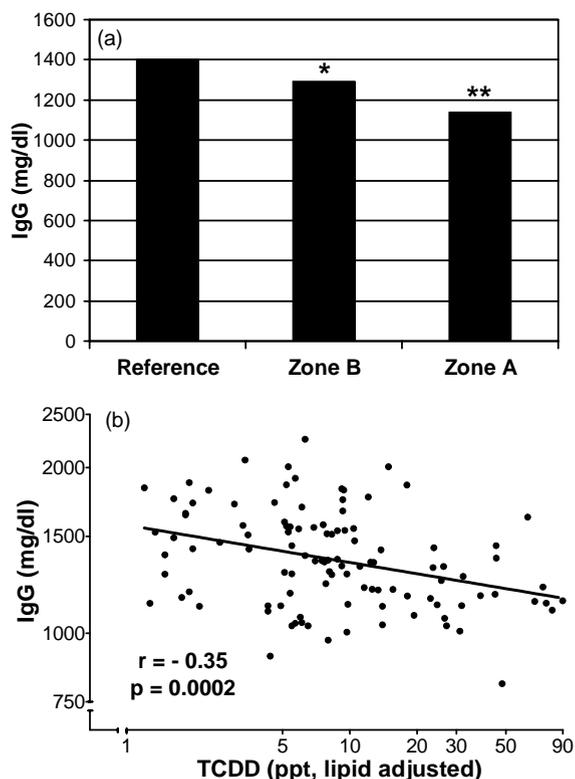


Fig. 1. IgG plasma concentrations and TCDD exposure in a random population sample of the highly contaminated zones (A and B) and of the surrounding non-contaminated reference area (non-ABR zone) of Seveso, Italy (from Baccarelli et al., 2002). (a) Plasma IgG were lower in subjects from zone B (* *P* = 0.03 vs. reference) and zone A (** *P* = 0.01 versus reference), the most contaminated area, than in the reference non-ABR zone. (b) Plasma IgG was negatively correlated with plasma TCDD levels (both variables are presented on a log-scale).

tors that may have biased the results were considered and excluded (Baccarelli et al., 2002). IgM, IgA, and complement component C3 and C4 plasma concentrations did not exhibit a consistent association with TCDD plasma levels (Baccarelli et al., 2002).

3.2. AhR-dependent markers in different cell conditions

In uncultured cells, only AhR, CYP1B1 and ARNT expression was analyzed. Mean mRNA levels per microgram total RNA were 1.19×10^6 copies for AhR, 0.47×10^6 copies for ARNT and 0.11×10^6 copies for CYP1B1 (Fig. 2).

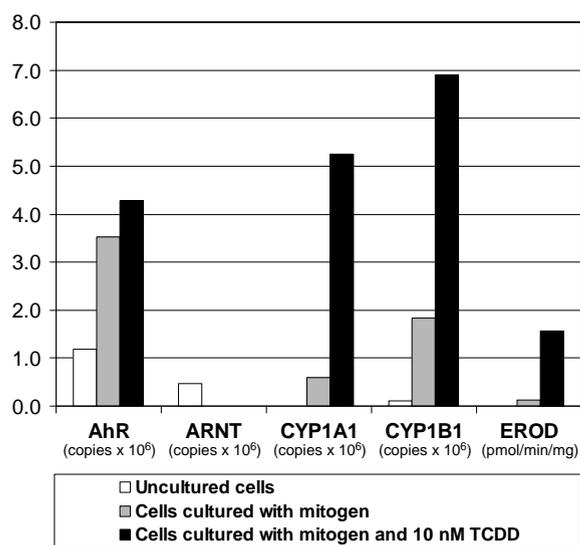


Fig. 2. AhR-dependent gene expression and EROD activity (geometric means) in uncultured cells and in cells cultured with mitogen or with mitogen + 10 nM TCDD from a random population sample of the highly contaminated zones (A and B) and of the surrounding non-contaminated reference area (non-ABR zone) of Seveso, Italy. Subjects with low pre-culture viability (<75%) were excluded from the analysis on uncultured cells. Data from Landi et al. (2003).

We did not measure ARNT expression in cultured cells because too few cells were available for the assay. In addition, ARNT has been shown to be poorly inducible in culture. All markers, including AhR, CYP1A1 and CYP1B1 expression and EROD activity, were highly induced when cells were cultured with mitogen and with mitogen + 10 nM TCDD (Fig. 2). As expected, when 10 nM TCDD was added to the mitogen-stimulated cells, CYP1A1 mRNA levels and EROD activity were the markers that showed the largest induction (8.8- and 13.1-fold, respectively).

3.3. Plasma TCDD and the AhR pathway

AhR (in all cell conditions) and ARNT (in uncultured cells) mRNA levels tended to be lower in subjects with higher TCDD exposure in the univariate analysis (Landi et al., 2003). In mitogen-stimulated cells, CYP1A1 expression was higher in subjects with higher TCDD levels (Landi et al., 2003).

We used multivariate statistical models to adjust the analysis for host and laboratory factors that may

have biased the results. Fig. 3 shows the main results of the multivariate analyses and the hypothesized relations between the biomarkers. The negative association between TCDD plasma levels and AhR was statistically significant in uncultured lymphocytes ($t = -2.28$; $P = 0.03$, model adjusted for age, gender, actin and date of assay). TCDD was not significantly associated with ARNT ($P = 0.21$) and CYP1B1 ($P = 0.60$) expression. In cells cultured with mitogen and in vitro TCDD, the association between TCDD plasma levels and AhR, CYP1A1 or CYP1B1 expression was not significant (after adjustment for actin expression, post-culture viability, experiment group and cell growth). Plasma TCDD levels were negatively and significantly associated with EROD activity ($t = -2.61$; $P = 0.01$) (Fig. 3).

3.4. Association among markers within the AhR-dependent pathway

In uncultured cells, we found a strong correlation between AhR and ARNT gene expression ($t = 4.20$; $P < 0.001$), AhR and CYP1B1 expression ($t = 4.50$; $P < 0.001$) and ARNT and CYP1B1 expression ($t = 2.26$; $P = 0.03$) in the multivariate model adjusted for age, gender, actin and date of assay (Fig. 3).

Markers measured in mitogen-treated cells and markers measured in cells cultured with mitogen + TCDD were strongly correlated ($P < 0.0001$). AhR and CYP1B1 expression levels measured in uncultured cells were not significantly associated with the corresponding AhR and CYP1B1 expression levels in TCDD-stimulated cultured cells ($r = 0.01$; $P = 0.93$ for AhR and $r = 0.14$; $P = 0.22$ for CYP1B1). Within cells cultured with mitogen + TCDD, AhR expression was highly and significantly associated with that of the CYP1A1 ($P = 0.001$) and CYP1B1 ($P = 0.006$) genes in the multivariate model adjusted for actin expression, post-culture viability, batch of experiment and cell growth. In addition, a marginal positive association between AhR expression and EROD activity ($P = 0.06$) was observed. As expected, CYP1A1 expression was highly correlated with the corresponding EROD activity ($P = 0.001$).

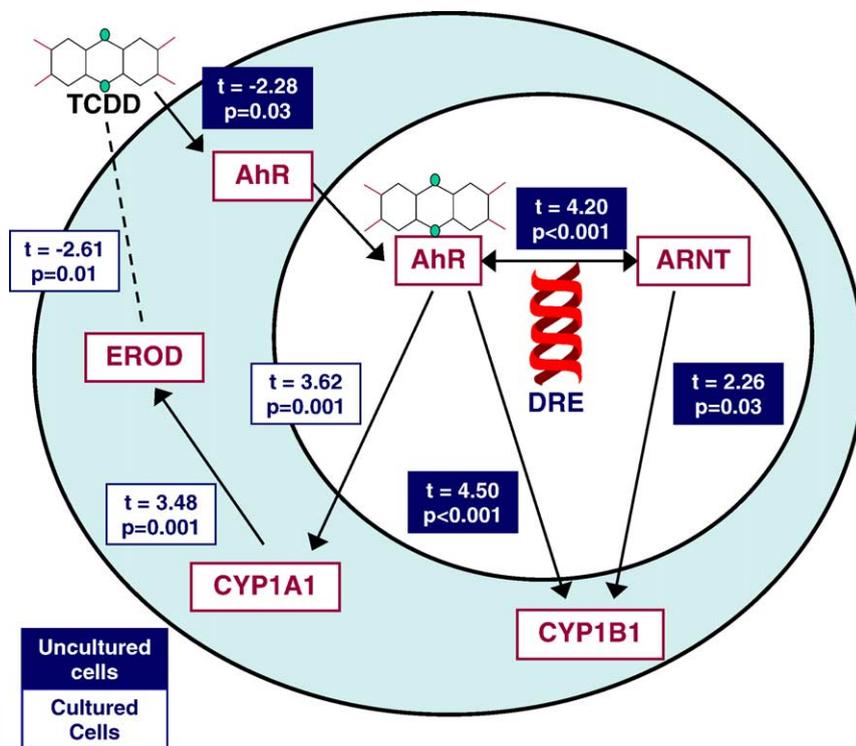


Fig. 3. Association between plasma TCDD and AhR-dependent markers in uncultured cells (black boxes) and in cells cultured with mitogen and 10 nM TCDD (white boxes) from a random population sample of the highly contaminated zones (A and B) and of the surrounding non-contaminated reference area (non-ABR zone) of Seveso, Italy. In order to express the association between markers, Wald's test t - and P -values from multiple regression analyses adjusted for age, gender, actin expression and date of assay (uncultured cells) or actin expression, post-culture viability, experiment group and cell growth (cultured cells) are shown. Data from Landi et al. (2003).

4. Discussion

TCDD is considered a potent carcinogen based on in vitro experiments and animal studies and exerts most of its toxic effects through the AhR.

The results of our study suggest that long-term presence of dioxin in the human body does not result in an increase in AhR pathway responsiveness or that responsiveness is eventually lost or reduced decades after the initial acute exposure. We are currently evaluating whether polymorphic variants of the CYP1A1 or CYP1B1 gene, as well as the corresponding estimated haplotypes, modify the association between dioxin body burden or TCDD added in vitro and p450 gene expression or EROD activity.

In the present study, we measured the expression of the genes in the AhR pathway and EROD activity in peripheral lymphocytes. While TCDD has effects on

diverse cell types, lymphocytes are readily available from blood. Mitogen-stimulated lymphocytes express the AhR, CYP1A1 and CYP1B1 genes and similarities in the regulation of lymphocyte CYP1A1 with the liver isoenzyme have been found, suggesting that CYP1A1 expression in peripheral blood lymphocytes can be used to monitor hepatic enzyme activity (Dey et al., 2001).

Experimental studies have shown that TCDD disrupts the differentiation of B lymphocytes, inhibiting the progression of pre-B cells to B lymphocytes and causing the suppression of antibody production (Holsapple et al., 1991). We found that plasma IgG concentration were lower in subjects exposed to TCDD after the Seveso accident. In addition, an excess of lymphoproliferative cancers was observed in cohorts of subjects exposed to dioxin (IARC, 1997), including this population (Bertazzi et al., 2001).

Peripheral lymphocytes may provide the best feasible surrogate for lymphatic cell populations for epidemiological investigations of TCDD toxic effects.

We carefully considered possible source of confounding and bias. We identified in previous studies (Landi et al., 1997, 1998) the determinants of TCDD levels in the population, such as age, gender, distance from the accident site, consumption of domestic livestock and poultry, and smoking. Many laboratory-related factors, such as pre- and post-culture cell viability, storage and shipment conditions, cell growth, day of experiment and actin expression, were strongly associated with gene expression and EROD activity. All these variables were considered as possible confounders in the planning, execution and analysis of the study.

The findings reported and discussed in this paper suggest the presence of long-term effects in the subjects exposed to TCDD after the Seveso accident. The mortality and cancer incidence follow-up of this population is continuing and will provide additional valuable information on dioxin risk. We are currently evaluating additional endpoints, possibly related to the risk of lymphoproliferative or other diseases, such as the frequency of chromosome rearrangements in peripheral lymphocytes and the pattern of gene expression and protein levels. We are planning to conduct a nested case-control study on the Seveso cohort to study the characteristics of and risk factors for non-Hodgkin lymphoma. In addition, the study of the subjects who developed chloracne, the well-defined acute toxic effect of TCDD, could produce critical results to better elucidate the dose–response profile, mechanisms of action and susceptibility factors contributing to dioxin toxicity in humans.

References

- Baccarelli, A., Mocarelli, P., Patterson Jr., D.G., Bonzini, M., Pesatori, A.C., Caporaso, N., Landi, M.T., 2002. Immunologic effects of dioxin: new results from Seveso and comparison with other studies. *Environ. Health Perspect.* 110, 1169–1173.
- Bertazzi, P.A., Pesatori, A.C., Bernucci, I., Landi, M.T., Consonni, D., 1999. Dioxin exposure and human leukemias and lymphomas. Lessons from the Seveso accident and studies on industrial workers. *Leukemia* 13 (Suppl. 1), S72–S74.
- Bertazzi, P.A., Consonni, D., Bachetti, S., Rubagotti, M., Baccarelli, A., Zocchetti, C., Pesatori, A.C., 2001. Health effects of dioxin exposure: a 20-year mortality study. *Am. J. Epidemiol.* 153, 1031–1044.
- Birnbaum, L.S., Tuomisto, J., 2000. Non-carcinogenic effects of TCDD in animals. *Food Addit. Contam.* 17, 275–288.
- DeVito, M.J., Birnbaum, L.S., Farland, W.H., Gasiewicz, T.A., 1995. Comparisons of estimated human body burdens of dioxinlike chemicals and TCDD body burdens in experimentally exposed animals. *Environ. Health Perspect.* 103, 820–831.
- Dey, A., Parmar, D., Dayal, M., Dhawan, A., Seth, P.K., 2001. Cytochrome P450 1A1 (CYP1A1) in blood lymphocytes evidence for catalytic activity and mRNA expression. *Life Sci.* 69, 383–393.
- Grassman, J., Landi, M.T., Masten, S., Spencer, D., Consonni, D., Edler, L., Needham, L., Caporaso, N., Mocarelli, P., Lucier, G., 1999. Determinants of ethoxyresorufin-*O*-deethylase (EROD) activity in human peripheral blood lymphocytes challenged in vitro with dioxin. *Organohalogen Comp.* 44, 375–379.
- Holsapple, M.P., Snyder, N.K., Wood, S.C., Morris, D.L., 1991. A review of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced changes in immunocompetence: 1991 update. *Toxicology* 69, 219–255.
- IARC, 1997. Polychlorinated dibenzo-*para*-dioxins and polychlorinated dibenzofurans. IARC Monogr. Eval. Carcinog. Risks Hum. 69.
- Landi, M.T., Needham, L.L., Lucier, G., Mocarelli, P., Bertazzi, P.A., Caporaso, N., 1997. Concentrations of dioxin 20 years after Seveso. *Lancet* 349, 1811.
- Landi, M.T., Consonni, D., Patterson Jr., D.G., Needham, L.L., Lucier, G., Brambilla, P., Cazzaniga, M.A., Mocarelli, P., Pesatori, A.C., Bertazzi, P.A., Caporaso, N.E., 1998. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin plasma levels in Seveso 20 years after the accident. *Environ. Health Perspect.* 106, 273–277.
- Landi, M.T., Bertazzi, P.A., Baccarelli, A., Consonni, D., Masten, S., Lucier, G., Mocarelli, P., Needham, L., Caporaso, N., Grassman, J., 2003. TCDD-mediated alterations in the AhR-dependent pathway in Seveso, Italy, 20 years after the accident. *Carcinogenesis* 24, 673–680.
- Masten, S.A., Grassman, J.A., Miller, C.R., Spencer, D.L., Walker, N.J., Jung, D., Edler, L., Patterson Jr., D.G., Needham, L.L., Lucier, G.W., 1998. Population-based studies of dioxin responsiveness: individual variation in CYP1A1 levels and relationship to dioxin body burden. *Organohalogen Comp.* 37, 13–16.
- Patterson Jr., D.G., Hampton, L., Lapeza Jr., C.R., Belser, W.T., Green, V., Alexander, L., Needham, L.L., 1987. High-resolution gas chromatographic/high-resolution mass spectrometric analysis of human serum on a whole-weight and lipid basis for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Anal. Chem.* 59, 2000–2005.
- Whitlock, J.P., 1999. Induction of cytochrome P4501A1. *Annu. Rev. Pharmacol. Toxicol.* 39, 103–125.