

Review Article

# The Effects of Dioxin on Reproduction and Development

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**Abstract:** The developmental effects of dioxin are important because of the high sensitivity of mammals as well as the irreversibility and longevity of the effects. In animal experiments, exposure to dioxin during pregnancy and lactation induce various functional effects on offspring at very low doses. In humans, even if there is no exposure to dioxin after birth, there might be effects on thyroid function in infants exposed to dioxin from breast milk. In this report, low-dose developmental effects of dioxins on offspring in animal experiments and human studies were reviewed. In terms of risk assessment, methods to describe dosimetry, models to describe dose-response and approaches to express health risk are discussed.

**Key words:** Dioxin, TCDD, Reproductive effects, Developmental effects, Risk assessment, Breast milk

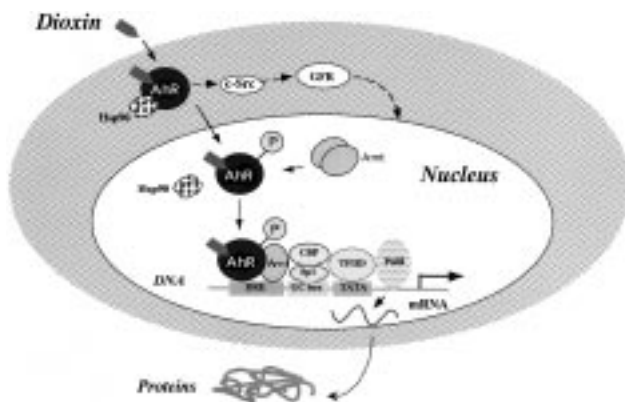
## Introduction

There has been great concern over environmental pollution involving dioxin. Worries over the adverse health effects of dioxin have been fuelled by reports of contamination around municipal incinerators and industrial waste sites and also of the exposure of employees at these facilities. The toxicity of dioxin is extremely high, and its effects on reproduction and development are being paid close attention, because the developmental process is highly susceptible to dioxin and the effects are often irreversible. In 1998, the World Health Organization (WHO) revised its tolerable daily intake (TDI) for dioxin, lowering the level from 10 pg/kg/day to 1–4 pg/kg/day<sup>1</sup>. This reevaluation was based on endometriosis in Rhesus monkeys and developmental effects in perinatally exposed offspring. The former TDI was based on data concerning carcinogenicity of dioxin, while the new one is based on recent studies on developmental effects. In other words the new TDI is a reflection of the growing evidence that reproduction and development are highly sensitive to the influence of dioxin.

## The Mechanism of Dioxin's Action and the Developmental Process

Most, if not all, of the effects of dioxin are mediated through binding to a cytosolic protein known as the aryl hydrocarbon receptor (AhR)<sup>2,3</sup>. AhR is a ligand-activated transcription factor, which forms a heterodimer with the aryl hydrocarbon receptor nuclear translocator (ARNT). This heterodimer interacts with dioxin-responsive enhancer elements (DRE) that are located upstream of target genes and activates their transcription. According to recent studies, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most potent prototype of dioxins, can exert its effects also in the cytosol through AhR-associated protein kinase, c-Src, to alter the functions of many proteins through a cascade of protein phosphorylations<sup>4</sup>. A schematic model of the action of dioxin is shown in Fig. 1. The receptor-mediated nature of the action mechanism is characteristic of hormones, growth factors and cytokines. Dioxin treatment leads to biological amplification of Ah receptor-mediated responses via a cascade of growth factors and hormones<sup>5</sup>.

Developmental processes are highly integrated; precise



**Fig. 1. A schematic model of the action of dioxin.**

Binding of the ligand (dioxin) to the aryl hydrocarbon receptor (AhR) results in the release of Hsp90 and translocation to the nucleus followed by dimerization to the aryl hydrocarbon receptor nuclear translocator (ARNT). The AhR-ARNT heterodimer binds with specific *cis*-acting enhancer, called dioxin responsive element (DRE), that promote the activation of several genes. Dioxins can exert its effects also in the cytosol through AhR-associated protein kinase, c-Src, to alter the function of many proteins through a cascade of protein phosphorylations.

integration of the endocrine system is needed at every stage of development. Since many hormones play important roles at each stage, exogenous environmental chemicals that mimic, inhibit or modulate endogenous chemical messengers can influence development. Dioxin alters cell growth and differentiation by affecting homeostasis and hormone balance through modulation of enzyme induction, growth factors, hormones and their receptors<sup>6</sup>. TCDD acts in an estrogenic or anti-estrogenic manner depending on the tissues and developmental stage<sup>7,8</sup>. It can decrease or increase thyroxine levels<sup>9,10</sup>. It can also decrease or increase transforming growth factor  $\alpha$  (TGF $\alpha$ ) depending on types of tissues<sup>11,12</sup>. TCDD suppresses or enhances the expression of glucocorticoid and epidermal growth-factor (EGF) receptors, depending on the type of cells and tissue, and the stage of development<sup>13–18</sup>. The tissue-specific and developmental-stage-specific actions of TCDD can be better understood by considering it to be an endocrine disruptor<sup>5</sup>.

It is also possible to understand the action of TCDD from the viewpoint of the physiological role of AhR. The normal function of AhR has not been clearly delineated and a normal endogenous ligand has not been identified. However, data on the biochemistry and molecular biology of AhR as a transcription factor indicate that well-regulated and controlled pathways exist for AhR to mediate the transduction of biochemical signals for the control of a number of hormones and growth factors. AhR may be necessary for the normal

development of many tissues including those in the neuroendocrine system. Thus, an AhR agonist, such as TCDD, could affect the developing neuroendocrine system<sup>19</sup>.

## Lethal Effect / Teratogenesis

The toxicity of TCDD in experimental animals has been found to be greater in embryos than in dams. The timing of TCDD administration is important in the occurrence of lethality (the day of gestation on which dosing occurred is an important factor). For example, when 24  $\mu\text{g}/\text{kg}$  of TCDD was administered once to pregnant C57BL/6 mice on day 6 of gestation (GD6), the number of stillbirths increased. However, when administration took place on GD8, GD10, GD12 or GD14, there were no effects<sup>20</sup>.

One of the characteristics of dioxin toxicity is the great species difference. However, this difference is much smaller in embryos than in adults. For example, the 50% lethal dose of TCDD for hamsters ( $\text{LD}_{50}$ : 1157–5051 mg/kg) is more than a thousand times greater than that for guinea pigs ( $\text{LD}_{50}$ : 1 mg/kg)<sup>21</sup>, but the  $\text{LD}_{50}$  for hamster embryos is only 12 times higher than that for guinea pig embryos<sup>22</sup>. Lethal toxicity in embryos is in the order of Rhesus monkeys~guinea pig>rat~rabbit>hamster~mouse.

When TCDD is administered to pregnant animals, there are various species-specific effects, including thymic atrophy, subcutaneous edema, growth retardation and stillbirths. Teratogenic effects of dioxin are not common in mammals, and are species specific. Cleft palates are observed in mice with TCDD concentrations that are neither toxic to the mother nor to the fetus and are induced especially at a critical period, on GD11 and GD12, but not on GD14<sup>20</sup>. Dioxin also causes hydronephrosis and thymic atrophy in mice. Hydronephrosis can also be observed in rats and hamsters, and there is no critical window of susceptibility; it can be induced by lactational exposure<sup>23</sup>. Both hydronephrosis and thymic atrophy are brought on by altered differentiation and proliferation of epithelial cells in the respective tissues. In the case of cleft palates, dioxin inhibits the proper fusion of the palate by inhibiting the decrease in EGF levels and increase in TGF $\alpha$  levels that occur during normal development<sup>24,25</sup>.

Hydronephrosis is a more sensitive defect than cleft palate in mice. This is due to hyperplasia of the ureteric epithelium, leading to blockage of the ureteric lumen, which then results in destruction of the renal parenchyma by elevated back pressure of urine outflow<sup>17</sup>. Both hydronephrosis and cleft palate are considered to be mediated by an Ah receptor since they are induced by PCDD, PCDF, co-planar PCB and

chlorinated naphthalene congeners that bind to Ah receptors. In fact, neither cleft palates nor hydronephrosis are induced by TCDD in AhR-knockout mice. However, in hetero (+/-) mice, cleft palate appeared in 20% of the mice while there was no suppression of hydronephrosis. It can therefore be considered that different mechanisms exist for the onset of hydronephrosis and cleft palate, and that the role of Ah receptors is different between the two conditions<sup>26</sup>.

In another study, the occurrence of cleft palate in AhR-knockout mice could not be completely suppressed<sup>27</sup>. This discrepancy may be partly explained by the difference in methods used to attenuate AhR expression.

### **Developmental Effects on Offspring Exposed *In utero* and Lactationally**

Much attention has been paid to the effects of dioxin in offspring exposed in utero and lactationally, since the dose level required to elicit these effects is very low.

#### *Effects on reproduction*

Peterson's group studied intensively and extensively the reproductive effects of TCDD in perinatally exposed offspring. They reported adverse effects on the male reproductive system in offspring of pregnant Holtzman rats given a single oral dose of TCDD as low as 0.064  $\mu\text{g}/\text{kg}$  on GD15<sup>28-30</sup>. The EPA group conducted a follow-up study using Long Evans rats with a similar experimental protocol<sup>8, 31-33</sup>. The results were summarized as follows: perinatal TCDD exposure reduced anogenital distance, reduced weight of accessory sexual organs, reduced spermatogenesis, and caused demasculinization or feminization of male sexual behavior. The decrease in ejaculated sperm number was greater than the decrease in cauda epididymal sperm reserve and daily sperm production (DSP) in the testes. However, fertility was not affected in these male rats. The greater decrease in sperm number in the distal excurrent duct system than in DSP may be due to change in the sperm transit rate. Sommer *et al.* did not find a change in transit rate<sup>34</sup>, but Wilker *et al.* reported its enhancement in male rats exposed to TCDD perinatally<sup>35</sup>.

Mably *et al.* reported a decrease in serum testosterone concentration and feminization of LH secretory patterns<sup>28</sup>. However, Gray *et al.* found neither a significant change in serum testosterone concentration, nor changes in the number of androgen receptors in the seminiferous tubules, prostate or epididymis<sup>32</sup>. Among the accessory sex organs, the ventral prostate is uniquely sensitive to *in utero* and lactational TCDD exposure<sup>36, 37</sup>. In male rats exposed to TCDD perinatally

and castrated at age 63 days, the responsiveness of ventral prostate weight and protein content to testosterone was inhibited<sup>36</sup>. Although the concentration of testosterone and dehydrotestosterone in the ventral prostate was reduced transiently, the alteration in the ventral prostate, characterized by impairment of the epithelial budding process, could not be explained by reduced androgen concentration<sup>38</sup>. Perinatal TCDD exposure affected the androgen-regulated prostatic protein mRNAs<sup>39</sup>. These effects were considered to be not the direct one but the result of delayed prostate development.

To investigate the mechanisms involved in the demasculinized or feminized sexual behavior in male rat offspring perinatally exposed to TCDD, the estrogen receptor system in sexually dimorphic brain nuclei was investigated; the estrogen receptor system and estrogen are thought to play an important role in sexual differentiation of the CNS. TCDD exposure did not affect the estrogen receptor system in sexually dimorphic brain nuclei or the volume of these nuclei<sup>40</sup>.

In female rats exposed to TCDD perinatally, malformations in external genitalia were observed, such as cleft phallus and vaginal thread, and delay in vaginal opening. No effects on estrus cyclicity, ovary function or serum estradiol concentration were observed. Although fertility was not affected, untreated stud males had difficulty attaining intromission and took longer to ejaculate<sup>8, 33</sup>. When the pregnant Long Evans rats exposed to TCDD on GD8, female offspring had enhanced incidence of constant estrus cycle in female offspring and reduced fertility from second to fourth litter when monitored by continuous breeding<sup>32</sup>.

In contrast to Gray's results, Chaffin *et al.* observed tissue-specific regulation of estrogen receptors (ER) and decreased circulating estrogen levels in female offspring of Holtzman rats given 1  $\mu\text{g}/\text{kg}$  of TCDD on GD15<sup>41</sup>. This decrease in estrogen levels resulted in altered ER mRNA levels. Significant reduction of pituitary FSH $\beta$  mRNA was also observed. Despite the reduced estrogen levels, neither serum FSH nor LH concentration increased significantly. Results of perinatal TCDD exposure suggest that it does not act on serum gonadotropin. Reduced serum estrogen levels appear to result from direct or indirect action on the ovary at some point following androstenedione production. Histologically, diminution in the number of antral and preantral follicles of certain size-classes was observed. However apoptosis does not appear to be the underlying mechanism of TCDD action in these particular follicles<sup>42</sup>.

While TCDD exposure of the male reproductive function and female external genitalia on GD15 is generally more toxic than on GD8, treatment on GD8 has more effect on

the estrus cycle and fertility in female offspring<sup>32</sup>). A cross-fostering protocol showed that the most of the effects on offspring that were exposed perinatally to TCDD can be induced by *in utero* exposure only, with the notable exception of feminized male sexual behavior<sup>43</sup>).

The effects on male reproductive function induced by *in utero* and lactational exposure to TCDD were also studied in mice and hamsters. The rat is the most sensitive mammalian species to developmental effects of TCDD; the sensitivity of hamsters is about 1/10 that of the rat<sup>32</sup>), and the sensitivity of mice is about 1/500 that of the rat<sup>44</sup>). Species differences in sensitivity are smaller in fetal lethality than in adult lethality. However, there are again large differences in developmental function toxicity, and the order of sensitivity does not always agree with that of adult lethality.

The mechanism that underlies the alteration of reproductive function in offspring exposed perinatally to TCDD may be the incomplete sexual differentiation of the CNS. MacLusty *et al.* suggested that such alteration is probably not mediated by blocking the estrogen response, but may instead involve subtle developmental changes in other parts of the endocrine system, perhaps also affecting the feedback control of the adenocortical function<sup>45</sup>).

#### *Effects on thyroid hormone*

The thyroid hormone plays a major role in the development of the brain. During this developmental process, a lack or excess of thyroid hormone arising at specific stages can cause irreversible neurological damage. The predominant source of brain thyroid hormone in the fetus has been shown to be fetal serum thyroxine (T4), not triiodothyronine (T3), so that a decrease in T4 during brain development might bring about brain damage. It has furthermore been suggested that a control system for thyroid function is established in the prenatal period, and that any temporary changes in thyroid hormone levels during this time might cause irreversible changes in thyroid function<sup>46</sup>).

Children and animals exposed to dioxin or PCBs in the perinatal period can exhibit various neurological disorders including learning and memory disorders, hyperactivity and delayed acquisition of auditory startle. These disorders resemble those seen in children exposed to thyroid hormone deficiency *in utero* and/or in infancy. Therefore, even low levels of dioxin or PCB exposure during the perinatal period can greatly influence neurological development by the effects of these chemicals on thyroid levels in the developing brain<sup>46</sup>).

TCDD was administered orally to Sprague-Dawley rats (GD10-16) and then T4 and UDP glucuronosyl transferase (UDP-GT) levels were measured at weaning. T4 significantly

decreased (79.6%) and UDP-GT significantly increased at exposures of 0.1  $\mu\text{g}/\text{kg}/\text{day}$ <sup>47</sup>).

#### *Effects on the immune system*

Extensive evidence has been published that demonstrates that the immune system is a target for TCDD toxicity. Thymic atrophy is a common immunotoxic effect of dioxin in experimental animals. Perinatal exposure to TCDD leads to thymic atrophy and a suppression of cell-mediated immunity that is more severe and persistent than that caused by adult exposure, suggesting that events involved in the maturation of the immune system are particularly sensitive to TCDD<sup>48</sup>). The hematopoietic precursors that migrate to the thymus are double-negative CD4-CD8- cells. In the thymus, these double-negative precursor cells proliferate and differentiate into double-positive CD4+CD8+ cells, which then differentiate into single-positive cells, CD4+CD8- or CD4-CD8+ cells<sup>49</sup>). Perinatal TCDD exposure decreases the numbers of double-positive cells and skews single-positive cell differentiation into CD4-CD8+<sup>50,51</sup>). Alterations to the lymphocyte stem-cell population in the fetus and neonate were induced by gestational exposure to TCDD (10  $\mu\text{g}/\text{kg}$  on GD14), as evidenced by a significant reduction in the lymphocyte stem-cell-specific enzyme terminal deoxynucleotidyl transferase (TdT)<sup>48</sup>). Gestational TCDD exposure suppresses delayed-type-hypersensitivity (DTH) response in rat offspring at a very low maternal dose<sup>52,53</sup>). Suppression of the DTH response in the offspring of F344 rats given TCDD on GD14 is persistent through late adulthood, occurs at doses as low as 0.1  $\mu\text{g}/\text{kg}$  to the dam, and is more pronounced in males than females<sup>53</sup>). Although the mechanism for TCDD-induced thymic atrophy has not been fully elucidated, involvement of the p27(Kip1) gene in the inhibition of thymocyte proliferation has been suggested<sup>54</sup>). TCDD induced p27(Kip1) cyclin/cdk inhibitor Ah receptor-dependently by altering Kip1 transcription. Kip1 is also induced by dioxins in cultures of fetal thymus glands, concomitant with inhibition of thymocyte proliferation and severe reduction of thymocyte recovery. Kip1 expression is likely to be the mediator of these effects because thymus glands of Kip1-deficient mice are largely, though not completely, resistant<sup>54</sup>).

#### *Effects on physiological function*

Perinatal exposure to TCDD alters thermoregulatory control in rats and hamsters<sup>55,56</sup>). Successive studies have confirmed a nocturnal hypothermia in rats. However, the hypothalamic thermoregulatory centers may not be altered permanently; functional alterations in the neuroimmune and/

or thermoregulatory axes involved in fever were suggested<sup>57</sup>).

## Effects on Humans

Epidemiological studies dealing with dioxins and their effects on human reproduction have been conducted in Vietnam, where the dioxin-contaminated defoliant 'Agent Orange' was used; following the Seveso accident; in environmental pollution events; and in occupational exposure. Nevertheless, there are few reports clearly showing the effects of dioxins on humans.

A recently released report suggested a relationship between exposure and the risk of neural tube defects in the children of Vietnam veterans who participated in the 'Ranch Hand' operation, which used Agent Orange<sup>58</sup>.

A Vietnamese researcher has also reported the effects of dioxin exposure, due to the release of Agent Orange, on reproduction and development<sup>59, 60</sup>. Although many effects of exposure have been reported (miscarriages, stillbirths, malformations such as cleft palates, and high risk of abnormal pregnancy), there have as yet been no detailed descriptions of the epidemiological procedures. It is difficult to evaluate these results because of the lack of information on selection and reporting bias of the subjects, confounding factors, etc.

At Seveso there has been no known increase in the number of birth defects attributed to dioxin exposure. However, in the area with the highest levels of contamination, known as zone A, an unusual sex ratio (48 females and 26 males) among the children born between 1984 and 1987 was observed<sup>61</sup>.

Breast milk contains a high level of dioxins and PCBs, which has led to concern for possible health hazards in nursing infants.

In Japan, the relationship between the concentration of dioxins (PCDD/PCDF) and co-planar PCB in breast milk, subpopulations of lymphocytes and the thyroid function in

101 mother-child pairs were investigated<sup>62</sup>. Breast milk samples were taken 2 to 4 months after delivery and blood samples one year after birth. The mean concentration of dioxin and related compounds was 22.6 ppt TEQ (range 3.4–48.5)/fat. There was a significant correlation between TCDD-TEQ in breast milk and TSH levels in the plasma of newborns. There seemed to be a tendency towards a negative correlation with T4 but this was not statistically significant.

In the Netherlands, there is an ongoing, large-scale prospective follow-up study on the relationship between exposure to dioxin/PCB during fetal life and lactation and developmental effects on children<sup>63–65</sup>.

More than 400 mother-child pairs have been studied, half of them from Rotterdam, a highly industrialized area, and half from Groningen, a semi-urban area. Half of the children are breast fed (BF) and the others formula fed (FF). Samples of maternal plasma in late pregnancy, and umbilical cord plasma are analyzed for 4 PCB congeners. In the second and sixth week after delivery, breast milk samples are analyzed for 17 most abundant PCDD/F congeners and three planar PCB congeners. Children have been examined 2 weeks after birth, and at 3, 7, 18 and 42 months. Relationships between health effects on children and their perinatal background exposure to dioxin/PCB are summarized in Table 1.

Median plasma PCB concentration at 42 months was 3.6 times higher in the BF than in the FF<sup>66</sup>. Maternal and cord plasma PCB levels were negatively associated with birth weight and postnatal growth until 3 months of age<sup>67</sup>. Prenatal PCB exposure was associated with an adverse effect on neurological condition at 2 weeks after birth and at 18 months<sup>68, 69</sup>, but not at 42 months<sup>70</sup>. Planar PCB levels were correlated with a higher incidence of hypotonia at 2 weeks after birth. At 3 months, prenatal exposure was significantly associated with lower psychomotor scores.

Maternal and infants' thyroid hormone status was related

**Table 1. Summary of the Dutch PCB/Dioxin study**

Endpoints	Related to prenatal exposure	Related to postnatal (breast milk) exposure
Growth	reduced birth size lower growth rate up to 3 month	
Neurological condition	lower neurological optimality (2 wk, one month) hypotonia (2 wk)	hypotonia (2 wk)
Psychomotor condition	lower psychomotor scores (3 month) lower cognitive learning scores (42 month)	
Thyroid function		reduced T 4 level (2 wk) increased TSH level (2wk)
Immunological condition	lower monocyte and granulocyte count (3 month) changes in T cell surface markers (18, 42 month)	decreased allergic reaction higher prevalence of middle ear infections

to perinatal PCB and dioxin exposure. Infants exposed to higher dioxin-TEQ levels had lower plasma T4 and higher TSH levels at 2 weeks after birth. The thyroid hormone status of infants was related to their neonatal neurological examination<sup>71</sup>.

Maternal plasma PCB concentrations, but not lactational or current exposure to PCBs, were significantly associated with lower cognitive ability at 42 months<sup>72</sup>.

Regarding immunological effects, prenatal and postnatal PCB and dioxin exposure was associated with lower monocyte and granulocyte count at 3 months<sup>73</sup>. Prenatal exposure to PCBs and dioxins was associated with a change in the T-cell lymphocyte population at 18 and 42 months. Current PCB exposure was associated with a higher incidence of middle-ear infections and a lower prevalence of allergic reactions at 42 months<sup>65</sup>.

In summary, significant associations between perinatal background dioxin/PCB exposure and adverse effects on growth, immunological parameters, neurodevelopment and behavior were found. Most of these adverse effects were associated with prenatal rather than postnatal exposure via lactation.

## Risk Assessment

As mentioned earlier, the revision of the WHO TDI in 1998 was based on the effects of dioxins on reproduction and development in experimental animals, and these studies have led to adoption of the body burden approach instead of the usual method of multiplying the NOAEL (no observed adverse effect level) or LOAEL (the lowest observed adverse effect level) by the uncertainty factor. This change is based on the assumption that the body burden that elicits any effect is almost the same among different species. Thus, the body burden that corresponds to LOAEL is estimated. Then the daily intake needed to obtain this body burden in a steady state is calculated.

The biological half-life of dioxin differs greatly according to species (in rats and mice: 10 to 20 days; in humans: 7 to 10 years), so that the daily intake necessary to achieve the same body burden is quite different for each species.

When radioactively-labeled TCDD is administered to pregnant rats, the transfer rate to their fetuses and the body burden in the mothers' bodies were investigated<sup>74, 75</sup>. The relationship between the body burden and the LOAELs of endpoints was obtained by animal experiments using the *in utero* and lactational exposure protocol.

When TCDD was administered once, orally, on GD15 to Long Evans rats, a decrease in the number of sperm in male

offspring, deformed external genitalia in females and many other defects appear at doses higher than 200 ng/kg. Using radioactive TCDD, the concentration in the fetus 24 hours after administration of 200 ng/kg was found to be 13.2 ppt. Accordingly, it is estimated that the body burden of the fetus at the LOAEL was 13.2 ppt.

It is possible to achieve the same concentration in the fetus as mentioned above by subchronic TCDD exposure of 10 ng/kg/day starting before pregnancy. With subchronic exposure, the effects on the external genitalia in females are almost the same as from a single oral administration on GD15. However, no decrease in sperm count is observed in subchronic exposure. With subchronic exposure, the number of live fetuses decreases<sup>76</sup>. Thus, even though the body burden is the same from the two ways of dosing, the same effects are not obtained. In the future, it will be necessary to determine what kind of dosimetry would be better to describe the effects of dioxin.

With the revision of the TDI, a body burden corresponding to the LOAEL has been obtained. An approach to express health risk objectively using a mathematical model has also been attempted. By describing the dose-response relationship, one can mathematically determine the risk of a certain body burden or the body burden corresponding to a certain level of risk. There have been few epidemiological studies on non-carcinogenic effects of dioxins on humans. Effective doses and the form of the dose-response curve have been studied by applying the results of animal experiments on the non-carcinogenic effects of 2,3,7,8-TCDD to the Hill model applied by McGrath<sup>77</sup>. For example, the body burden that brings a risk in excess of 5% of reducing the sperm count in the testes is 2.02 ng/kg and the daily intake in humans that elicits the same body burden is 1.01 pg /kg/ day. It has been indicated that the parameter that expresses the form of the dose-response curve is less than 1. This suggests that the relation is linear and that there is no threshold<sup>78</sup>.

## Summary

The developmental effects of dioxin are important because of the high sensitivity of mammals as well as the irreversibility and longevity of the effects. In animal experiments, exposure to dioxin during pregnancy and lactation induce various functional effects on offspring at very low doses. In humans, even if there is no exposure to dioxin after birth, there might be effects on thyroid function in infants exposed to dioxin from breast milk. Officially, it is said that breast milk is safe<sup>79</sup>. However, there are still

many unknown factors regarding the effects of dioxins on growth and development. The revision of WHO's TDI in 1998 was based on the results of animal experiments on the effects of dioxins on reproduction and development. The new TDI was based on the body-burden approach, which was based on the idea that the body burden that elicits any effect is almost the same among species. However, it has been reported that even if the concentration among fetuses is the same, the toxicity may vary depending on the dosing method. In terms of risk assessment, further study is needed to discover a better dosimetry, using the body burden of the fetus or dam, or the concentration in a target organ. Mathematical models are highly useful because they can be used to define risk objectively and the relationship between dose and response can be easily described.

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