5. Effects on humans

The human endocrine system controls various biofunctions via complex mechanisms. Disrupting this system can result in a variety of health effects. Thus far, there have been no reports on definite causal relations between health effects on humans and endocrine disruptors, excluding instances caused by diethylstilbestrol (DES) which have been for possible pharmacological actions on the endocrine system.

On the other hand, investigations on wildlife and some epidemiological investigations on humans have revealed many types of diverse effects on the female reproductive system, male reproductive system, thyroid gland, hypothalamus, and pituitary gland. It is feared that these chemicals can generate negative effects on not only the parent generation directly exposed to them, but also the next generation. These effects include endometriosis, uterine cancer, breast cancer in women, and prostate cancer, testicular cancer, decreased sperm count, and hypospadias in men.

The paragraphs below outline these effects. However, there are many aspects left to elucidate, including causative substances, the environment, and mechanisms. If any relationship between these effects and possible endocrine disruptors are indicated, it will be necessary to conduct animal studies and to determine details such as the types of chemicals and the severity of effects, .

(1) Effects on the female reproductive system and mammary glands

1. Uterine cancer

   Uterine cancer can be divided into carcinoma of the uterine cervix and carcinoma of the uterine body, but the latter is closely related to hormones. There are approximately 12,000 cases of uterine cervix cancer a year in Japan (estimated for 1990), but thus is thought not to be closely related to hormones. There are approximately 3,000 cases of uterine body cancer a year in Japan (estimated for 1990), and although the incidence of uterine cervix cancer is decreasing, the age-standardized incidence of uterine body cancer is increasing. Internationally, however, the incidence of uterine body cancer in Japan is less than half that of the U.S. and Europe.

The risk factors of uterine body cancer are similar to those of breast cancer, and include duration of menstruation, childbirth and obesity, which affect endogenous hormone levels. In addition, estrogen supplementary therapy after menopause and female hormone compounding agents of estrogen-progestogen sequential use are known to increase the risk of uterine body cancer*[18]. In the U.S., the incidence of uterine body cancer after menopause increased from the latter half of the 1960's, but begun to decrease from the mid 1970's. It has been suggested that this decrease is linked to the spread of hormone supplementary therapy using only estrogen, and the switch to combination therapy with progestogen. In women who used drugs containing female hormones (i.e. estrogen and progestogen in combination), the risk reportedly decreased by about 50% compared to those who did not use the drugs*[18]. That is, when progestogen is administered concomitantly, the risk of uterine body cancer is decreased via the suppression of uterine gland cell proliferation. There is little literature on the effect of chemicals suspected of endocrine disruption, but most of these chemicals reduce the risk of uterine body cancer; as to dioxin, one theory holds that its effects are due to its anti-estrogen action*[19].

The administration of tamoxifen, which is known to reduce the risk of breast cancer, was found by randomized controlled trial to increase the risk of uterine body cancer*[20].

2. Endometriosis

The number of reported cases with endometriosis is on the increase due to the improvement of medical care technology and probably also due to actual increase in incidence. The causes of endometriosis remain to be unclarified, but are thought to be multi-factorial. Based on studies in humans, it is clear that estrogen is needed for the development and progress of endometriosis. However, it does not necessarily play a decisive role in the etiology of endometriosis in that no apparent abnormality in estrogen secretion is associated with endometriosis.

In a study using a small number of rhesus monkeys, the relation between exposure to dioxin and endometriosis has been suggested*[21]. However, a recent study using rhesus monkeys, indicated that polychlorinated biphenyl

(PCB) at as a ten times higher concentration, (standardized by dioxin) as that used in the previous study does not induce endometriosis*[22].

In humans, dioxin was reportedly detected in the blood of 8 of 44 infertile women with endometriosis, whereas it was detected in 1 out of 35 infertile women without endometriosis, indicating a higher dioxin detection rate in patients with endometriosis*[23]. On the other hand, Canadian research has recently reported no difference in plasma concentrations of total organic chlorine compounds, including dioxin, between 86 patients with endometriosis and 70 control cases*[24]. At this moment, information and knowledge are insufficient regarding any link between endometriosis and endocrine disruptors.

At present, a continuing study is under way in Seveso, Italy on women exposed to TCDD. Further research must be promoted to elucidate the relation between endocrine disruptors and endometriosis.

3. Breast cancer

Breast cancer occurs at highest incidence in women in their late 40's and in their 60's, the age distribution of breast cancer having two peaks. In Japan, there are approximately 27,000 cases a year (estimated for 1992), and the age-standardized incidence is increasing. The incidence of breast cancer is low in Japan, at less than half that of the U.S. and Europe. However, in Japanese who have moved to the U.S., breast cancer has increased at a higher rate than the comparable rate in Japan, which suggests its relation with radical changes in eating habits.

The risk factors for breast cancer include early menarche, late menopanse, late childbirth, nulliparity, obesity and diet (excess calories, few vegetables), factors that affect endogenous hormone levels. The increasing trend of breast cancer in Japan, and the regional differences seen internationally, can in part be explained by these factors. It has been reported that post-menopausal supplementary therapy using hormone preparations may increase the risk because of the delay of menopausal age for the period the estrogen is used, so a high concentration of estrogen in the body may be closely related to the development of breast cancer, by promoting proliferation of mammary gland

cells.

The generally used female hormone compound agents contain progestogen and estrogen, but not all researchers agree that they increase the risk of breast cancer. The amount of hormones, the type of hormone preparation used, period of use, when used (before or after menopause) and features of the user (family history of breast cancer, nulliparity) seem to have different effects. In regard to DES, which was used during the 1940's to prevent miscarriage, its etiological relation with vaginal clear cell adenocarcinoma during puberty and adolescence in daughters born from women using the chemical has been established, but the risk of breast cancer is reportedly increased by about 30% in the mothers themselves, as evidenced in randomized controlled trial participants and follow-up study on users*[25]. On the other hand, a recent randomized controlled trial has confirmed that in women without breast cancer, continuous administration (over several years) of the breast cancer treatment drug tamoxifen, which binds with estrogen receptors in competition with estrogen, decreases the risk of breast cancer*[20]. It has also been suggested that phytoestrogen contained in soy beans decreases serum estrogen concentrations and decreases the risk of breast cancer, which fact may be related to the small number of breast cancer cases in Japan*[26].

In regard to endocrine disruptors, since their main actions are estrogenic and anti-estrogen, it has been suggested that they may have some effect on the development of breast cancer. Various epidemiological studies have been conducted regarding the relation between breast cancer and exposure to chemicals suspected of endocrine-disrupting effects, such as chlorine pesticides (DDT and its metabolite DDE), dioxins (especially TCDD), which are combustion byproducts, and polychlorinated biphenyls (PCBs), which are industrial byproducts. Follow-up studies on women highly exposed to TCDD and PCBs professionally, and studies on comparison of DDE and PCB in fatty tissues and serum among patients and controls have been reported, but information and knowledge actively supporting their relation have not yet been obtained*[19].

As per the above, in regard to pharmaceuticals synthesized to have estrogenic actions in themselves, they may increase the risk of breast cancer relative to

dosage and period of use, but there is little evidence that environmental low dosage exposure to endocrine disruptors exerting estrogenic action is a risk factor for breast cancer in Japan; the possibility is thought to be low at present.

4. Others

It is known that exposure of a female fetus to DES increases the risk of congenital uterine anomaly, and the later development of vaginal cancer. DES is a strong synthetic estrogen preparation, and was used in the past as a drug to prevent miscarriage. It used to be administered to treat breast cancer, but presently is not clinically used for this purpose.

DES may be considered an endocrine disruptor in the broad sense, but although many other substances indirectly affect hormone actions, this is the strongest estrogen preparation; the above abnormalities due to this preparation are thought to result from receiving inappropriate estrogen exposure in utero. That is, this can be viewed as a pharmacological action of estrogen to the fetus, and not the specific action of DES. Among the chemicals occurring in the environment, no other substance has estrogenic property as strong as DES; since the prohibition of DES use, DES-associated abnormalities no more come about.

(2) Effects on the male reproductive system

Certain kinds of chemicals are reported to have estrogenic actions or anti-androgenic actions on experimental animals and to cause abnormalities in the male reproductive system, such as in reduced spermatogenesis ability and reproductive-duct abnormalities. These results from animal experiments invite concerns that these chemicals can have various adverse effects, such as a decline in sperm count and an increase in the incidence of prostate cancer in humans.

Several studies have been conducted on humans. Observations on males exposed to DES as fetuses have revealed that (1) disrupting actions of exogenous estrogen on the reproductive system during the developmental period were weaker than those in rodents, and (2) there was no rise in the incidence of cancer in the male reproductive system. Below are the suspected major health effects of chemicals on humans.

1. Decline in sperm count

In 1993, Sharpe and Skakkebaek*[27] reported in *The Lancet* their hypothesis that reproductive anomalies such as decline in sperm count, testicular cancer, hypospadias, prostate cancer and male infertility are increasing in relation to estrogen in the environment. In 1993, Colborn et al.*[28] also took up 63 chemicals regarded as having endocrine-disrupting actions similar to estrogen actions, and the number of such chemicals is increasing. Representative chemicals include DDT, PCBs, nonyl phenol used in synthetic detergents, bisphenol A and phthalic acid ester used in the plastic materials and plasticizers, styrene contained in Styrofoam, and dioxin, which is generated in the process of industrial production and in the incineration of garbage. Steroid hormones used as pharmaceuticals and natural phytoestrogen have also been listed as chemicals thought to exert endocrine-disrupting actions. In regard to their effects on human sperm, there have been reports on sperm count decline due to fetal DES exposure, professional exposure to pesticides and other substances, and dioxin exposure in veterans of the Vietnam War, but there have been reports disputing these as well*[29]*[30]. Moreover, all instances of established effects of endocrine disruptors on humans have involved accidental exposure. Currently, there are no reports of endocrine disruptors within the general living environment causing declines in human sperm count, decrease in the quality of semen or abnormalities in the reproductive organs.

In regard to the human sperm question, at present there is not enough information as to methodology, nor enough basic knowledge. Although there is a need for comparisons according to age, time changes and regional differences, even standards for seminal findings in healthy Japanese males have not been accurately established.

Under these circumstances, an international joint research project was organized in 1997 by Skakkebaek et al. to study the reproductive functions of normal males. Investigations are now under way in Denmark, Finland, Scotland, France, Japan and other countries, and are scheduled to begin in the U.S. this fall. In Japan, Iwamoto's group is conducting an investigation in

the Kawasaki-Yokohama region from November 1997. Investigations on regional differences are scheduled to begin in a number of areas all over Japan at the end of this year. Reproductive functions are the keys to survival of a species, not only humans but all living beings. Possible effects on reproductive functions, as long as they are suspected, should be regarded with the highest priority regardless of whether there is concrete evidence, and no matter what the cause might be.

a) Background of problems with sperm counts

In 1974, Nelson and Bunge of the U.S. reported that the average sperm count of 386 males before vasectomy for contraceptive purposes was unexpectedly low (48×10^6/ml) compared to that of cases reported before (more than 100×10^6/ml in many cases), and hypothesized that some kind of environmental factor was involved*[31]. MacLeod of Cornell University in the U.S., who was an authority on sperm*[32], criticized this study, and a report in 1979 on an investigation conducted on more than 15,000 males over a period of ten years concluded that there was no decreasing trend in sperm count. This settled the controversy at the time, and although there were subsequent reports on the decrease in sperm quality, this controversy did not reemerge until the 1990's.

Skakkebaek, on the other hand, who had been researching testicular cancer at the University of Copenhagen, learned from statistical investigations that the incidence of testicular cancer had increased by three to four times in Scandinavia during the past 50 years. From several researches, almost all cancer registries in the Western World have noted a remarkable increase in testicular cancer. Focusing on the relation of this finding with the reported increase of abnormalities in male reproductive organs, such as cryptorchidism and hypospadias, he began tackling this issue by organizing a team. To investigate whether semen quality has changed during the past 50 years, his group attempted to examine past scientific literature from around the world, to find data on sperm counts in healthy males. The results, reported in the *British Medical Journal* by Carlsen et al.*[33], were shocking, as it revealed that the human sperm count had halved in 50 years. Immediately after, many studies from various countries followed, reporting on the decline in sperm count and the decrease in semen quality. Not only were these studies

introduced in medical journals; the general mass media also rushed to take them up. The report by Carlsen et al. thus caused a sensation; but at the same time it was harshly criticized. Faults in the selection standards of the quoted literature and statistical analysis methods were pointed out, and actual investigations denying a decrease in semen quality followed. Recently, along with such negative reports, have come reports that there has indeed been a decrease in semen quality, so this controversy is still ongoing. At the same time, attempts are under way to reinvestigate this matter.

b) Reports concluding that sperm counts are decreasing

Carlsen et al. reported that the average sperm concentration decreased by 42%, from 113 $\times$ 10^6/ml in 1940 to 66 $\times$ 10^6/ml in 1990, and that the average semen volume decreased significantly, from 3.40 ml to 2.75 ml. Auger et al.*[34] reported that as a result of investigating 1,351 Parisian approved sperm donors aged 19 to 59 years from 1973 to 1992, sperm concentration decreased by 2.1% a year, from 89 $\times$ 10^6/ml in 1973 to 60 $\times$ 10^6/ml in 1992, that motile sperm count and with sperm count normal morphology also significantly decreased, and that in younger generations, sperm concentration, motility rate and normal sperm morphology rate decreased significantly. They concluded that in these males there was a decrease in semen quality. Van Waeleghem et al.*[35] reported from Belgium, in an investigation over the past 19 years on the semen of 416 candidate sperm donors aged 20 to 40 years, that sperm concentration decreased by 12.6 $\times$ 10^6/ml, but that there was no change in sperm count per ejaculation. Furthermore, when the periods 1977 - 1980 and 1990 - 1995 were compared, the normal sperm morphology rate was seen to decrease from 39.2% to 26.6% and forward sperm motility rate to decrease from 52.7% to 31.7%. Irvine et al.*[36] compared before 1959 and after 1970 in Scotland, and indicated that sperm concentration declined from 98 $\times$ 10^6/ml among donors born before 1959 to 78 $\times$ 10^6/ml among donors born after 1970. Ginsburg et al.*[37] compared the periods 1978 - 1983 and 1984 - 1989, and reported that sperm concentration decreased from 101 $\times$ 10^6/ml to 96 $\times$ 10^6/ml.

c) Reports indicating that sperm concentrations are not declining but rising

In 1979, MacLeod et al.*[32] studied the time-related change in sperm

concentration from 1966 to 1977, but as there was no decreasing trend in sperm concentration or seminal fluid volume, concluded that sperm concentration had not decreased since 1951 when they gave their first report. Fisch et al.*[38] conducted a comparative study of three areas in the U.S. during the period from 1970 to 1994, and found that sperm concentration and motility rate were 72.7 × 10^6/ml and 51.4%, respectively, for California, which was the lowest, followed by 100.8 × 10^6/ml and 56%, respectively, for Minnesota and 131.5 × 10^6/ml and 58.2%, respectively, for New York, which was the highest. Their research showed that there were significant differences among the three areas, but concluded that the sperm count over the past 25 years had not decreased in these areas. Paulsen et al.*[39], in an investigation in Seattle, reported that there was no decline in sperm count from 1972 to 1993. Bujan et al.*[40] reported no decline in sperm concentration during a 20-year period from 1972 to 1992 in the Toulouse, France. A report from Israel in 1997*[41] concluded that there was no change in sperm concentration and motility rate from 1980 to 1995.

d) Reports from Japan

The first report in Japan on normal male sperm count was that by Takashima et al.*[42] in 1954, when it was 57.6 × 10^6/ml. In 1982, Yoshida*[43] reported, based on an investigation for two years from 1976, that the average seminal fluid volume was 3.0 ml and that the average sperm concentration was 106 × 10^6/ml, excluding one case of azoospermia. In 1984, Ikegaki et al.*[44] reported that sperm concentration was 70.9 × 10^6/ml, based on an investigation for five years from 1975. Recently, Oshio et al.*[45] reported that in a study on those in their 20's and 40's, the average sperm concentration was 45.8 × 10^6/ml and 78.0 × 10^6/ml, respectively, and that the average motility rate was 27.2% and 28.0%, respectively, and pointed out the decline in sperm count and motility rate for those in their 20's. Iwamoto et al. reported in research on partners of pregnant women that the average sperm concentration was 82.6 × 10^6/ml and motility rate was 54%, and Yoshimura et al.*[46] reported that time changes in seminal findings indicated a decline in sperm count, but further analysis is awaited.

e) Problems regarding human sperm studies

Seminal findings can change pathologically, as well as physiologically, due to various factors. In much of the past literature, the abstinence period was not constant. The collection of semen through masturbation is affected by various problems, such as repetition. When semen was submitted a number of times, there have been studies that used only the first results or the best of several results. Results may also change according to the technical expert conducting the test. Measurements by automatic sperm analyzers vary according to the equipment, which is not a problem with relative changes within the findings, but data obtained using different equipment cannot be strictly compared.

Fisch*[38] reanalyzed the regional diversity of the data by Carlsen et al.*[33], and showed that the average sperm concentration differed greatly according to region, and that much of the research done initially contained much data from New York, which had a high concentration, suggesting that regional inclinations had disproportionately increased the levels of early data. It has been often reported that data from New York show high sperm concentration, but the reason for this is not known. In regard to changes in sperm count, before considering whether or not there is a real decline worldwide, it is necessary to focus on the changes in a specific area. This is because semen quality may be decreasing in one area but not in another. The problem of regional differences is often regarded as a factor distorting analysis results, but the fact of regional differences in semen quality is in itself an interesting phenomenon. The question of regional differences must be addressed from the aspect of genetic factors, as well as environmental factors.

2. Prostate cancer

Prostate cancer develops in men over 50 years old; there are approximately 9,000 cases a year (estimated for 1992) in Japan. The age-standardized incidence tends to be increasing, but internationally the incidence in Japan is about 10 to 20% that of the U.S. and Europe. In Japanese who have moved to the U.S, the incidence has clearly increased, and its relation with changes in dietary habits has been pointed out. The radical increase in incidence seen from the latter part of the 1980's in the U.S is thought to be due to the increase in number of cases discovered by the spread of prostate cancer screening methods, such as serum PSA (prostate-specific antigen), and by

palpation; prostate cancer incidence is recently decreasing. There are no established risk factors for prostate cancer, but factors that affect the levels of endogenous hormones (testosterone, etc.), such as diet (high intake of fats and red meats, low intake of vegetables) and sexual activity have been suggested, and it is thought to be a hormone-related cancer.

In regard to prostate cancer's relation with chemicals suspected of having endocrine-disrupting actions, a follow-up study of a small number of people professionally exposed to such chemicals, as for example, by spraying of herbicides, or from coke oven exhaust, showed a risk increase*[47], but there has been little epidemiological research and there is little information compared to the amount available concerning breast cancer.

3. Testicular cancer

Testicular cancer develops in young males in their 20's to 30's, and it has a peculiar age-related characteristic. In Japan, the incidence is approximately 800 patients a year (estimated for 1992), and although the age-standardized incidence is based on a small number and is unstable, it shows a tendency to increase. In the U.S. and Europe, it formerly tended to increase in all countries, but recently has leveled off or is decreasing. In the U.S., the incidence differs among ethnic groups by about five times, with no increase seen for black males, who have a low incidence. The incidence in Japan is remarkably low compared to that of the U.S. and Europe, as with other hormone-related cancers. As the risk factors of testicular cancer, cryptorchidism related to low androgen conditions has been established, and reportedly increases risk by about two to ten times*[48]. The relation with material estrogen preparation use during early pregnancy has been reported in a small number of cases, but in a follow-up study of 250 boys born of pregnant women receiving DES or placebo, there was no case of testicular cancer in either group*[30].

There are not many reports on testicular cancer's relation with chemicals suspected of endocrine-disrupting actions. Some studies show that while the disease is increasing in incidence in Scandinavian countries, DDE concentration in breast milk has been decreasing since around 1970, and that

despite similar levels of DDE in breast milk for the four Scandinavian countries, there are regional differences in testicular cancer incidence, with a factor of four*[49]. In light of these studies, it is thought that there is little relation between DDT and testicular cancer.

4. Hypospadias

Hypospadias is a congenital anomaly of a relatively high incidence among urological diseases in boys. It originates in incomplete fusion of the labioscrotal fold in morphogensis of the external genitalia of the fetus from about 8 to 12 weeks, and results in an abnormal opening of the urethra in the ventral penis or scrotum. Development of the external genitalia depends on the testosterone (T) secretion of the fetal testicles.

The cause of this condition is still unknown, but it is thought to be due to the interaction of factors including those of the mother, such as first childbirth or late childbirth; environmental factors, such as fetal exposure to mutagens, and genetic factors. Clinical research has shown that there are cases in which the patients lack T metabolites and T receptors. Hypospadias is thought to result from abnormalities of endocrine that affect the development of male reproductive organs.

Reports on trends in the incidence of hypospadias show that the condition tended to increase in the 1980's compared to the 1970's in Norway*[50], Sweden*[51], Denmark*[52], the U.K.*[53] and Hungary*[54]. According to the report by Matlai et al.*[53], it increased in England and Wales; in Hungary*[51] it increased from 12 to 24 per 10000 births in a comparison between 1971 and 1983. In Sweden, Kallen et al.*[55] reported that hypospadias increased from 8 to 12 per 10000 births from 1965 and 1979, but this increase occurred from 1969 to 1973, with no increase observed after 1973; the investigation method has not been changed in this study. In an investigation by the International Clearinghouse for Birth Defects Monitoring Systems*[56] until 1993, hypospadias tended to increase in Denmark and Norway, while an increase was not indicated in the U.K., Sweden and Hungary. The European Registration of Congenital Anomalies has reported that in a study of 17 regions in Europe from 1980 to 1992, an increasing trend was seen in the

Strasbourg and Malta regions, while a decreasing trend was seen in Northern Netherlands and the Glasgow and Liverpool regions. In Australia*[57], hypospadias has tended to gradually increase from 1985, with regional differences based on statistics for 1990 to 1992, with 21.5 per 10,000 births. In 1997, Paulozzi et al.*[58] analyzed the trend in hypospadias based on remarkably detailed data by the Metropolitan Atlanta Congenital Defects Program and the Birth Defects Monitoring Program of the U.S., and found that the incidence in 1993 was about two times that in 1968, which was significantly higher, and that the increase rate was 2.9% a year during this period, with an increase of 5.7% per year among nonwhites other than that of 1.4% per year among whites. In particular, severe hypospadia, in which the opening of the urethra is in the scrotum or perineal region, was reported as increasing by 2.7 times in 1990 and by 5.5 times in 1993 compared with 1967. In the U.S., the increase rate was high in the order: West, Central, Northeast and Southeast regions. On the other hand, the trend in Japan is increasing slightly, according to the results of congenital anomalies monitoring by the Japan Association of Obstetricians & Gynecologists (JAOG); the incidence is 2 to slightly less than 3 per 10,000 births in recent years, which is low compared to that of other countries.

Studies in the 1970's showed that fetal exposure to contraceptives, pregnancy test drugs or progesterone used for continuation of pregnancy led to hypospadias, and was related to the increase in incidence. Sharpe et al.*[27] have suggested that the over twofold increase in developmental disorders of male reproductive organs in the past 30 to 50 years may be due to the changes in dietary habits, increase in endogenous estrogen due to increase in body fat, increase in intake of dairy products, or the effects of estrogenic chemicals in the environment. Goodman et al.*[59] have revealed from results of experiments on fetal rats that exposure to progesterone causes hypospadias. On the other hand, in 1995 Raman-Wilms et al.*[60] reported no relation between such exposure and anomalies of the external genital organs. The aforementioned report from the U.S. indicated that severe hypospadias is increasing, which was not reported before, but the causes have not been reported; the report only stresses the importance of study on the risk factors.

From the above literature, there are data indicating that synthetic

progesterone exposure is related to the incidence of hypospadias in animal experiments, but at present there seems to be no evidence that endocrine disruptors cause hypospadias in humans.

5. Effects on other male reproductive organs

To briefly explain the development of male reproductive organs, at 7 weeks, fetal sex-determining region (SRY) genes become active and produce the testis-determining factor (TDF). As a result, Sertoli precursor cells develop in the primitive reproductive glands, and anti-müllerian hormone (AMH) is secreted. Furthermore, fetal Leydig interstitial cells are induced, and the secretion of testosterone (T) begins. During the period when the ureteral bud develops at the base of the wolffian duct, the müllerian duct develops parallel to the wolffian duct. In males, the müllerian duct is immediately degenerated by AMH. By the 9th week, the external genitals consist of the genital wall surrounding the genital tubercle, genital ridge and urogenital sinus. By the 12th week, the testicles descend to near the deep inguinal ring. At around that time, the left and right genital wall and genital ridge fuse to make the cavernous body of the urethra and scrotum, and the genital tubercle enlarges and becomes the penis glans. After 7 months, constriction of the testis gubernaculum begins due to the effects of T and other androgens, and by 9 months, the testicles reach the base of the scrotum*[61].

In this differentiation of the reproductive organs, testosterone (T), active type DHT and their receptors and 5α-reductase in the androgen target cells play an important role. If a disorder occurs in any of these processes, reproductive organ anomalies occur.

Cryptorchidism is another abnormality that is relatively often observed along with hypospadias. Cryptorchidism can be classified into four grades according to the position of the testicles, which includes the high grade, which remains in the abdominal cavity, and the low grade, which is at the high scrotum. Severe hypospadias is said to often be accompanied by the complication of cryptorchidism. There are many theories as to the cause of the complication; a number of factors are thought to be related. Reportedly son from women treated with DES from 1945 to 1971 often develop

cryptorchidism and hypospadias, as well as decrease in reproductive functions. According to exposure data on experimental animals and humans, high concentrations of DES exposure cause disorders in the male reproductive organs, including cryptorchidism, but the effects at low dose exposure have not been clarified. A relation between estrogen concentration in the mother and the incidence of cryptorchidism has also been indicated. As these facts are apparently related to the increase and decrease in reproductive organ developmental abnormalities in the past 40 to 50 years, Sharpe et al.*[27] report that these abnormalities may be caused by estrogen exposure during fetal development.

Many fetal period factors are known to affect the incidence of cryptorchidism upon birth, including bodyweight at birth, gestational age and number of fetuses. The manifestation of cryptorchidism is also reportedly influenced by seasonal changes as well as racial differences. (The incidence of cryptorchidism is clearly low in black males compared to white males, as is the incidence of testicular cancer.)*[62]

In regard to the trend in the incidence of cryptorchidism, in a study of 7,500 boys born in Oxford, England, comparison of cryptorchidism incidence between the mid-1950's and 1980's showed an increase from 4.0% to 5.4% at birth and from 0.96% to 1.86% at three months after birth*[63]*[64]. In contrast, according to recent research on 6,935 boys born at Mt. Sinai Hospital in New York, the incidence of cryptorchidism was 3.7% at birth and decreased to 1.0% at 3 months of age. As the rate was not significantly different from that several decades ago, it was concluded that there is no evidence of an increase in cryptorchidism*[65]. However, this result may be due to the apparent increase, which may have been observed because there is no standardized diagnosis method or patient identification method, or because of regional differences. There are no data clearly indicating an increase in cryptorchidism in Japan, as epidemiological investigations are difficult to conduct, due to problems with diagnosis.

In regard to abnormalities in the another external genitals, such as decrease in penis size, these can occur during any developmental stage of the reproductive system, in the presence of factors that damage androgen actions,

but they rarely occur alone; and their relations with endocrine disruptors are unknown.

(3) Effects on the thyroid system

Thyroid gland secretes thyroxin (T4) and triidothyronine (T3), hormones important for both physical and mental development. Thyroid is the gland most studied regarding the effects of endocrine disruptors next to glands. PCB and dioxin excreted into the environment accumulate in fish, and also in birds, marine mammals and humans according to the predatory linkage. When PCBs enter the body of animals during embryonic stage and infancy through maternal blood or breast milk, they decrease the supply of T4 to the nervous system by binding with transthyretin, which is the T4 transport protein in the blood[*66*67*68*69]. They also cause deficiency of thyroid hormones delivered to the central nervous system by promoting the hepato-biliary excretion of thyroid hormone into the intestines[*70]. In the brain, they increase the deiodinase, which induces T3, an active thyroid hormone from T4[*70] in situ. When given to infant animals[*71] they inhibit the activity of an enzyme called chorine acetyltransferase. Animal experiments have indicated that this action is inhibited by T4 administration but not by T3.

Regarding humans, there have been reports from the U.S. and Europe of a relation between the consumption of fish contaminated with PCBs and the index indicating the degree of immature nervous development in infants[*72*73*74*75*76]. Regarding whether it is caused directly by actions on thyroid hormones, 105 pair of newborn babies and their mothers were studied in pairs in Netherlands. It was found that mothers who had breast milk with high PCB concentrations had significantly low concentrations of T4 and T3, and that blood TSH concentrations were significantly high in the babies[*74]. In Japan and in Taiwan, "yusho" occurred, an accident in which PCB was mixed in edible oils. It has been reported in a study from Taiwan that children born of "yusho" mothers have a slightly delayed cognitive capability even after seven years[*77]. The relation with thyroid hormone metabolism during nervous system development has been speculated. However, there have been no reports that blood thyroid hormone levels decrease significantly in exposed adults or babies. There is some advocate the condition to be similar to that of thyroid hormone refractriness[*78*79], although any elusive evidence to support this notion has not been available. There has been a report

that thyroid functions decrease as a result of professional exposure in PCB plants, but the causes of thyroid abnormalities are autoimmune thyroid diseases, so the relation with PCBs is not known[*80].

(4) Others

1. Effects on the hypothalamus and pituitary gland

   There is no concrete evidence as to their effects on the hypothalamus and pituitary gland.

   Effects on the immune system have been indicated particularly in TCDD and PCB exposure. One of the receptors that mediate these effects is assumed to be aryl hydrocarbon (Ah) receptor.

   Apprehensions regarding learning disabilities and psychological disorders were particularly an issue in relation to PCB exposure, but there are no uniform research results at present.

2. Effects on wildlife

   It has been reported by the United States that environmental chemicals have had damaging effects on the reproductive functions of alligators and several marine invertebrates in Florida. For example, a relationship was indicated between a chemical spill (dicofol) into Lake Apopka in Central Florida, and shrunken penises and decreased hatching ratios for male alligators in the lake[*81]. Also, it was hypothesized[*82] that a change in the sex ratio of herring gulls (fewer male) in the Great Lakes resulted from estrogenic substances, such as DDT in the environment. In Japan, imposex (i.e., male sexual characteristics appearing in females) is reported on gastropods (IBONISHI, REISHIGAI). The induction of imposex is known to be caused by exposure to tributyltin (TBT) compounds, which are used as antifouling paints on ships.[*83]

   Although several effects on wildlife have been reported, thorough research should be conducted to evaluate effects on humans, considering the differences in susceptibility to endocrine disruptors between animal species.

(5) Evaluation of health effects on humans

The secretion and metabolism of hormones are controlled in the body in a complex way. The mechanism that control the changes in the hormones is thought to be

subject to a constant condition regulated by an adjusting mechanism, so-called "homeostasis," including feedback control of hormone concentrations.

Therefore, as to the effects of endocrine disruptors on humans 1) many such chemicals have little binding affinity to receptors; 2) their concentrations in the environment are generally low, so some doubt that they will precipitate adverse reactions. Excluding DES, which has strong reactivity and was administered directly to humans in large amounts, it is thought that, for now, necessary measures can be taken to limit daily exposures, provided that the risks and exposure level's of each substance can be accurately evaluated. However, for these measures to succeed, the following pre-conditions must be met: 1) Physiologocal conditions must not be such that hormone-controlled disruption could be easily caused, including during the fetal period, 2) Unexpected synergistic effects must not be caused by multiple chemicals, and 3) There must be no unknown responsive morphologies, which could not ensure dose dependability in a low-dose response.

The results of animal experiments suggest that there may be adverse effects of endocrine disruptors on human health. Therefore, it should be kept in mind that although possible health effects on humans may not be excessive, the chemicals may have some health effects. In light of this, careful investigations and evaluations must be conducted. In particular, as regards the effects on the fetus, it is necessary to undertake comprehensive investigations, taking into consideration irreversible responses whose patterns may differ from those of adults.

Concerning those diseases whose causal relations with exposure to endocrine disruptors appear to have been demonstrated (including endometriosis, breast cancer, and developmental abnormalities of male reproductive organs), so far, there is no detailed information supporting such causal relations.