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REVISION AND ESTABLISHMENT OF JAPANESE DRINKING WATER QUALITY GUIDELINES FOR DI(2-ETHYLHEXYL) PHTHALATE, TOLUENE AND VINYL CHLORIDE – DIFFERENCES FROM THE LATEST WHO GUIDELINE DRAFTS –

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ABSTRACT — The revision of the Japanese drinking water quality guidelines was established in May 2003. The WHO drinking water quality guidelines for the 3rd edition were also revised and the draft has been open to the public since last year. Most guideline values of each chemical in both Japan and WHO were quite similar; however, there are different overt values for three chemicals. In this short communication, we describe them and discuss the reason for taking the different toxicity endpoints and derivation method for these three chemicals, di(2-ethylhexyl) phthalate, toluene and vinyl chloride.

KEY WORDS: Drinking water quality guidelines, Di(2-ethylhexyl) phthalate, Toluene, Vinyl chloride

INTRODUCTION

The revision of the Japanese drinking water quality guideline was established in May 2003 and implemented on May 2004. In this revising, regulated chemical lists were modified because of the past detection trend or exposure prospect. The chemicals already listed in the previous version were reevaluated and chemicals newly listed in this revision were assessed with the latest toxicity information. The Japanese guidelines derivation has referred to the concurrent WHO revision, and both of the general principles for the guidelines (GD) derivation are almost the same. Although most guideline values of chemicals in Japan were similar to those of WHO, some minor differences between WHO and Japan exist because of different default body weight application for the guideline calculation (50 kg/Japan vs. 60 kg/WHO). Furthermore, in some cases, different drinking water contribution ratios (allocation) to total exposure media were used for the guideline values calculation from tolerable daily intake (TDI) on account of the regional chemical exposure assessment. These differences were not owing to the difference of health risk assessment per se. However, the different guideline values for di(2-ethylhexyl) phthalate (DEHP), toluene and vinyl chloride between the Japanese guidelines revision (2003) and the latest rolling revision of WHO drinking water quality guideline were mainly caused by the health risk assessment variation. In this short communication, we describe the reason for taking the different toxicity endpoints or derivation method of the guidelines. Table 1 shows the guideline values for three chemicals of the WHO 2nd edition (WHO, 1996) established in 1994 and rolling revision in 2003, and previous and present Japanese versions.

DERIVATION OF GUIDELINE VALUES

Di(2-ethylhexyl) phthalate (DEHP)

As the guideline value of DEHP by the WHO 2nd edition, 0.008 mg/L was derived from a no observed

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adverse effect level (NOAEL) of 2.5 mg/kg/day in a rat feeding study (Morton, 1979) for 7 days according to no induction of hepatic peroxisome proliferation. The hepatic tumors were considered to be the most critical endpoint and hepatic peroxisome proliferation to be closely related to the carcinogenic mechanism. An uncertainty factor of 100 was applied only because of the animal most sensitive to peroxisome proliferation, and the allocation of 1% that was used as DEHP is generally not contained in food (WHO, 1996). For the latest WHO assessment, the guideline value of DEHP was not changed from the 2nd edition, because it was not listed for the detailed reevaluation.

In 1994, the Japanese government decided to use the same data and derivation method for domestic drinking water guidelines except for 10% allocation and 50 kg instead of 60 kg for human body weight. The guideline value was 0.06 mg/L.

However, the Japanese government established a TDI for DEHP in 2001 when high contamination was found in some specific foods and the health risk was deeply concerned (Koizumi *et al.*, 2001). In this assessment, TDI ranging from 40 to 140 μg/kg/day was established from a NOAEL of 3.7 mg/kg/day for testicular toxicity in a rat study (Poon *et al.*, 1997) and 14 mg/kg/day for reproductive toxicity in a mouse study (Lamb *et al.*, 1987), respectively, applying an uncertainty factor of 100 for intra- and interspecies differences. As for hepatic peroxisome proliferation, it was taken out for extrapolation to humans because IARC (2000) concluded that the hepatic tumor due to DEHP in rodents (in association with peroxisome proliferation) is not relevant to other animal species including humans (Group 3). Although it is clearly shown that there are strong species differences in testicular toxicity such as severely toxic in rats and guinea pigs, weakly in mice but not in hamsters, marmosets and cynomolgus monkeys, the potential of testicular toxicity in humans cannot be excluded at this moment. Therefore, the guideline of 0.1 mg/L was derived from 40 μg/kg/day of TDI using 10% of allocation, and 2 L of daily water intake for 50 kg body weight of the Japanese population.

Toluene

In 1994, WHO tried to re-assess the toxicity data of toluene and made the same conclusion as the previous value, 0.7 mg/L. A TDI of 0.223 mg/kg/day was derived using the lowest observed adverse effect level (LOAEL) for marginal hepatotoxicity in mice of 312 mg/kg/day (equivalent to 223 mg/kg/day, as there were 5 days per week) (NTP, 1990) and applying an uncertainty factor of 1,000 (100 for inter- and intra-species variation and 10 for the short duration of the study and use of a LOAEL instead of a NOAEL). This TDI yields a guideline value of 0.7 mg/L (rounded figure), allocating 10% of the TDI to drinking-water (WHO, 1996).

The Japanese government used the same data and derivation method for the domestic drinking water guideline except for 50 kg instead of 60 kg for human body weight. The guideline value was established as 0.6 mg/L in 1994.

For the new revision, the Japanese Government used a different toxicity endpoint, neurotoxicity, which is the most typical toxicity for toluene. In the case of neurotoxicity with histopathological changes as well as carcinogenicity and developmental toxicity without maternal toxicity, some additional uncertainty factors should be considered to derive a TDI. Toluene showed neuropathological effects in the brain consisting of neuronal cell necrosis in the dentate gyrus and Ammon's horn of the hippocampus at 1250 and 2500 mg/kg/day. NOAEL for neurotoxicity was 625 mg/kg/day (equivalent to 446 mg/kg/day, as there were 5 days per week) and a TDI of 0.0892 mg/kg/day was derived by application of an uncertainty factor of 5,000 including additional uncertainty factors of 5 for short exposure duration and 10 for neuropathological changes. This TDI yields a guideline value of 0.2 mg/L (rounded figure), allocating 10% of the TDI to drinking-water.

Table 1. Comparison of three guideline values (mg/L) between WHO and Japanese drinking water.

	WHO Guideline		Japanese Guideline	
	1994 (2nd ed.)	Revising 2003 (3rd ed.)	1994	2003
DEHP	0.008	0.008*	0.06	0.1
Toluene	0.7	0.7	0.6	0.2
Vinyl chloride	0.005	0.0003	No setting	0.002

*: No detailed reevaluation draft.

Vinyl chloride

It has been generally accepted that a mathematical model such as a linearized multistage is appropriate to estimate a low-dose cancer risk of a genotoxic carcinogen. There is sufficient evidence showing that vinyl chloride is a multiple site carcinogen and its metabolites are genotoxicants. Table 2 shows the incidences of hepatic tumor-related lesions in studies reported by Feron *et al.* (1981) and Til *et al.* (1991).

In the WHO 2nd edition, a linearized multistage model was applied to the incidence of angiosarcomas in female rats which was reported by Feron *et al.* (1981) only because of a good relationship with the human incidence at that time. An excess cancer risk at 10^{-5} was 0.010 mg/L. The guideline value was 0.005 mg/L, applying an uncertainty factor of 2 for double risk by exposure from birth (WHO, 1996).

On the other hand, in the WHO rolling revision, total liver tumors (angiosarcomas, hepatocellular carcinomas and neoplastic nodules) from the same study are incorporated to derive the guideline value including conversion to human equivalent doses (using the physiologically based pharmacokinetic (PBPK) model of U.S. EPA, 2000, Clewell *et al.*, 2001). A linear low-dose extrapolation was conducted by drawing a straight line between 10% of the low estimate dose (Benchmark dose approach) and the origin (zero dose). The results were nearly identical with those derived using the linearized multistage model. The concentrations in drinking-water of 0.0005 mg/L were calculated as being associated with excess risks of liver tumors of 10^{-5} for lifetime exposure beginning at adulthood. Exposure from birth would double this risk (U.S. EPA, 2000). This would result in a rounded guideline value of 0.0003 mg/L for a theoretical risk of 10^{-5}.

The guideline for vinyl chloride was not set in the previous Japanese guideline.

As described in Table 2, Feron *et al.* (1981) obtained clear evidence of carcinogenicity in rat liver in a three-dose setting study but the low dose of 1.7 mg/kg/day was still carcinogenic in female rats. The same group (Til *et al.*, 1991) conducted a further study up to 0.014 mg/kg/day and showed that the middle dose of 0.13 mg/kg/day was a non-carcinogenic dose. As both studies had been conducted under mostly the same experimental conditions, these data would be considered from a single study with doses ranging 1,000 times. For derivation of the newly established

Table 2. Summary incidence of hepatic tumor-related lesions for two rat carcinogenicity studies conducted by the same group.

	Til *et al.*, 1991				Feron *et al.*, 1981			
mg/kg/day	0	0.014	0.13	1.3	0	1.7	5.0	14.1
Male								
Neoplastic	0/99[a]	0/99	0/99	1/49	0/55	1/58	7*/56	23*/59
nodules	(0)[b]	(0)	(0)	(2.0)	(0)	(1.7)	(12.5)	(39.0)
Hepatocellular	0/99	0/99	0/99	3*/49	0/55	1/58	7*/56	23*/59
carcinoma	(0)	(0)	(0)	(6.1)	(0)	(1.7)	(12.5)	(39.0)
Angiosarcomas	0/99	0/99	0/99	1/49	0/55	1/58	2/56	8*/59
	(0)	(0)	(0)	(2.0)	(0)	(1.7)	(3.6)	(13.6)
Female								
Neoplastic	0/98	0/100	1/96	9*/49	2/57	26**/58	39*/59	44*/57
nodules	(0)	(0)	(1.0)	(18.4)	(8.8)	(44.8)	(66.1)	(77.2)
Hepatocellular	1/98	0/100	1/96	3/49	0/57	4*/58	19*/59	29*/57
carcinoma	(1.0)	(0)	(1.0)	(6.1)	(0)	(6.9)	(33.2)	(50.9)
Angiosarcomas	0/98	0/100	0/96	2/49	0/57	0/58	2/59	9*/57
	(0)	(0)	(0)	(4.1)	(0)	(0)	(3.4)	(15.8)
Total liver					2/57	28/58	49/59	56/57
tumors[c]					(8.8)	(48.2)	(83.1)	(98.2)

[a]: Number of lesion-bearing animals / number of analyzed animals.

[b]: Percentages of incidences.

[c]: The total number of animals with tumors derived from US IRIS(2000) / number of analyzed animals.

Statistically significant compared to the controls with *$p<0.05$ or **$p<0.01$ was reported in the original articles.

Japanese guideline value, the neoplastic nodules were not taken into account for the following reasons. As there was no diagnosis of nodular hyperplasia in those reports, there is a possibility that the neoplastic nodules may include not only hepatocellular adenoma but also nodular hyperplasia, which is not considered to be a neoplastic leision. The high incidence of neoplastic nodules at 1.7 mg/kg/day in females quickly dropped to less than half at 1.3 mg/kg/day and virtually no incidence at 0.13 mg/kg/day. This dose-response may not be appropriate for extrapolation to low doses. The incidence slope of total liver tumors mostly reflected the high incidence of neoplastic nodules rather than the real cancer incidence. In addition, because hepatocellular carcinomas and angiosarcomas originate from different cells, liver and vascular cells respectively, the evaluation of combined incidences may draw a conflicting conclusion. Therefore, the dose-response incidences of hepatocellular carcinoma in female rats were considered to be most appropriate for application to dose-response analysis, in view of data from the two reports. After dose conversion based on the PBPK model, an excess risk of 10^{-5} by the multistage model was calculated to be 0.0875 mg/kg/day as a virtual safety dose (VSD). The guideline of 0.002 mg/L was derived using 2 L of daily water intake for 50 kg body weight of the Japanese population. The allocation factor was not applied for the mathematical model approach because of large uncertainty caused by highly lower dose extrapolation.

DISCUSSION

Table 3 summarizes the derivation processes of all three chemicals. Although the detailed reevaluation draft for DEHP has not been published in the 3rd WHO water quality guideline, it was presumed that the derivation process would be same as the 2nd edition because were no changed guideline values. The general principle for the derivation of TDI and VSD is the same between Japan and WHO; however, the difference in the choice of critical endpoints leads to varied guideline values. In the Japanese assessment, testicular toxicity of DEHP and neurotoxicity of toluene were used to derive a TDI instead of their hepatotoxicity adopted by WHO. In the case of vinyl chloride, the same critical study was used for the guideline derivation, but the adopted neoplastic endpoints were differ-

Table 3. Summary of guideline value derivation in WHO (3rd ed.) and Japan (2003).

endpoint	NOAEL (mg/kg/day)	uncertainty factor: inter-species	uncertainty factor: intra-species	uncertainty factor: use of LOAEL	uncertainty factor: study period	uncertainty factor: nature of toxicity	TDI or VSD* (mg/kg/day)	allocation (%)	body weight (kg)	water consump. (L)	guideline value (mg/L)
DEHP(WHO)†											
hepatic peroxisome proliferation	2.5	10	10				0.025	1	60	2	0.008
DEHP(Japan)											
testicular toxicity	3.7	10	10				0.04	10	50	2	0.1
Toluene(WHO)											
hepatotoxicity	223	10	10	10			0.223	10	60	2	0.7
Toluene(Japan)											
neurotoxicity	446	10	10		5	10	0.0892	10	50	2	0.2
Vinyl chloride(WHO)											
total liver tumors (angiosarcoma, hepatocellular carcinoma and neoplastic nodules)											0.0003¶
Vinyl chloride(Japan)											
hepatocellular carcinoma							0.0875*		50	2	0.002

†: Derived from the 2nd edition.

¶: At the initial calculation from experimental animal data, the guideline concentration of 0.0005 mg/L was derived at 10^{-5} excess risk concentration during adulthood. Then the concentration was decreased to half because of doubled risk for exposure from birth.

*: Virtual safety dose corresponding to an excess cancer risk of 10^{-5}.

ent from each other because of the different interpretation on the cancer risk assessment. The adverse effects in experimental animals for the human health assessment are chosen by consideration of appropriate extrapolation to humans, which is expected from the nature of the toxicity, toxicity mechanism, etc. With regard to taking appropriate toxicity endpoints for derivation, the latest Japanese decision is considered to be more suitable on the basis of recent scientific consideration as described before. Because the revisions for the 3rd edition of water quality guidelines in the WHO are still ongoing, the assessment and the guideline value may be changed until the fixed version is published.

As for the derivation of the guideline value from the TDI, the estimation of the exposure contribution ratio (the allocation) is another important issue. In the case of DEHP, both levels of TDIs or NOAELs estimated in Japan and WHO are similar, although the critical endpoints are different. The guideline values were different at one order of degree from each other, because the allocation factor for drinking water of the TDI estimated in WHO was one-tenth of that in Japan. The allocation depends on environmental circumstances as well as chemical physical properties, and local exposure assessment is necessary for the estimation of the allocation factor of the respective chemical. Although the DEHP exposure contribution for drinking water in the WHO 2nd edition was estimated to be considerably lower, the allocation of 10% was applied in Japan as the default value when the exposure assessment was not elucidated.

Given the risk management of drinking water supplied by the Waterworks, the derivation of the guideline values of chemicals may be a regional issue. However, a large amount of drinking water bottled as mineral water has been circulating worldwide and the regulated values of chemicals will also be based on the drinking water guideline. Therefore the need for the international harmonization of chemical risk assessment will be required even more in the future.

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生殖発生毒性を指標としたダイオキシンの耐容1日摂取量（TDI）算定の考え方について

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The recent TDI derivation of the dioxin based on the reproductive and developmental toxicity.

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SUMMARY

In 1998, WHO-IPCS re-assessed the TDI of dioxin, which was derived from the body burdens of TCDD exposed to the experimental animals. Then, the international assessment agencies and governmental assessment agencies have conducted the dioxin fs health assessment using the similar method to the WHO-IPCS approach. The key endpoints were reproductive and developmental toxicity caused by *in utero* and lactational exposure. Each assessment agencies used the similar data set of the toxicity studies, however, there are some differences about the TDI derivation method and the selection of adverse endpoints. This report reviewed the recent reproductive and developmental toxicity studies of the dioxins and summarized the health assessment in the international or governmental agencies, and discussed the appropriate TDI derivation.

Key Word: dioxin, tolerable daily intake, reproductive and developmental toxicity

はじめに

1998年にWHO-IPCSが，ダイオキシンの体内蓄積性を考慮して体内負荷量という概念を用いて，耐容1日摂取量（TDI）の再評価を行った[1]．我が国でも1999年に，体内負荷量を基にしてTDIの設定を行っている[2]．これ以前は，発がん性を感受性の高いエンドポイントとしてTDIを設定していたが，この体内負荷量という物差しを用いることにより，胎児期及び授乳暴露による次世代への影響がより感受性の高い毒性指標となることが明らかになった．その結果，これ以降はヨーロッパ各国やJECFAなどの評価機関では，この評価法に従い耐用摂取量評価を算定してきている．本研究では，この評価法の重要なエンドポイントであるダイオキシン類による生殖発生毒性に関して，1998年以降の新しい知見と，各国および国際評価機関でのTDI算定経過をまとめると共に，現時点での適切なTDIのあり方について考察した．

体内負荷量とTDI

まず，体内負荷量を用いたTDIの算定法について概略を示す．一般に化学物質の耐用摂取量は，最も感受性の高い毒性学的エンドポイントを基に算定される．ヒトを対象とした定量的で信頼性の高い疫学研究などの知見がある場合はそれを用いるが，通常は疫学上の交絡因子を完全に排除することは難しく，動物実験における化学物質の投与用量を基に，ヒトにおける耐用摂取量を算出している．1990年代の前半頃まではダイオキシンに関して最も感受性の高い毒性は，げっ歯類に対する発がん性であると考えられており，評価機関の多くは，ラットを用いた2年間の長期投与試験における無毒性量（NOAEL）：1ng/kg/dayを基に，不確実係数（多くは100）を適用して耐容1日摂取量を設定していた．しかし，1998年のWHO-IPCS[1]での評価以後は，ダイオキシン類のように脂溶性が高く，排泄が遅い物質は，長い時間をかけて徐々に体内に化学物質が蓄積していくことや，ヒトとラットでは数百倍も排泄速度が異なることから，投与量と蓄積濃度（＝体内負荷量）と関係はヒトとラットで著しく異なることになり，投与量をベースにして毒性発現を比較するのは適当ではないと判断された．また，ダイオキシン類による毒性発現は蓄積量である体内負荷量に依存して発現していることが示され，近年は，この体内負荷量を基に定量的な毒性評価を行うようになった．この体内負荷量という物差しを用いることにより，従来の発がん性よりも，胎児期及び授乳期暴露による次世代への影響がより感受性の高い毒性指標となることが明らかになった．この際，摂取量と体内負荷量との換算は，1コンパートメントモデルの定状状態における，以下の近似式で示すことができる．

摂取量(ng/kg/day)＝[体内負荷量(ng/kg) X ln(2)]／[半減期(day) X吸収率]

また，ヒトや実験動物に関する様々な知見より，ヒトと実

験動物では同じ体内負荷量で毒性が発現すると判断されている．したがって，TDIを求めるには，まず動物実験において最も低い体内負荷量で発現するエンドポイントを選定し，その体内負荷量においてはヒトでも同様の影響が現れると仮定する．その後，上記の式からその体内負荷用に相当するヒトにおける1日摂取量を算出し，この値に対して不確実係数を適用して，TDIを算定するという手順を踏むことになる．

ダイオキシン類による生殖発生毒性

1998年以降に公表された2,3,7,8-四塩化ジベンゾパラジオキシン(TCDD)の生殖発生毒性に関する論文の内容について検討した(Table 1)．

SDラットの妊娠15日に200または1000 ng/kgのTCDDを単回強制経口投与したところ[3]，両TCDD投与群で母体体重が低下し，1000 ng/kg投与群では出生児数の減少がみられたが，雄児の成長にはTCDD投与の影響は観察されなかった．雄児の生後30日において1000 ng/kg投与群で17-ヒドロキシラーゼ活性及び精巣上体重量の低下，生後45日に精巣の3 β-及び17 β-ヒドロキシステロイドデヒドロゲナーゼ，5 α-リダクターゼ活性上昇及び血清中アンドロゲン濃度の低下が認められた．

Holtzmanラットの妊娠15日に250 ng/kgのTCDDを単回強制経口投与した[4]ときには，TCDD投与の雄児に精巣上体精子数の減少，前立腺重量の低下が観察されたが，肛門生殖突起間距離（AGD），性成熟，精巣の1日精子産生，精巣重量及び前立腺を除く副生殖器重量にTCDD投与の影響は認められなかった．

Holtzmanラットの妊娠15日に1000 ng/kgのTCDDを単回強制経口投与した[5]ところ，投与後4日の雌胎児でミュラー管尾部の間充織の肥厚，ミュラー管癒合の阻害，ウヲルフ管退行の阻害が観察され，これらが永久的なvaginal thread(膣糸)の原因であることが示唆された．

Long Evansラットの妊娠15日に1000 ng/kgのTCDDを単回強制経口投与し[6]，雄児の精嚢について調べた．雄児体重及び精嚢重量には生後32日までTCDD投与群と対照群との間に差はみられなかったが，49日以降ではTCDD投与群において低下が認められた．TCDD投与群では精嚢上皮の分岐及び分化を低下させることによって，精嚢の発生を傷害することが示唆された．

Syrianハムスターの妊娠11.5日に2000 ng/kgのTCDDを強制経口投与した[7]ところ，母体の生存率及び体重，児数にTCDD投与の影響はみられなかったが，TCDD投与群のF1児の体重増加抑制，膣開口遅延，膣性周期の変化が認められた．雌F1では陰核の完全分裂が観察されたが，vaginal threadは観察されなかった．雌F1を無処置の雄と交配させたところ，TCDD投与群で妊娠率低下し，妊娠ハムスターの死亡率上昇，分娩生存児数減少，離乳率低下が観察された．これらの結果から，妊娠母体に有害な作用を示さない投与量のTCDDが雌児の成長，繁殖機能，生殖器の形態に悪影響を及ぼすことが明らかになった．

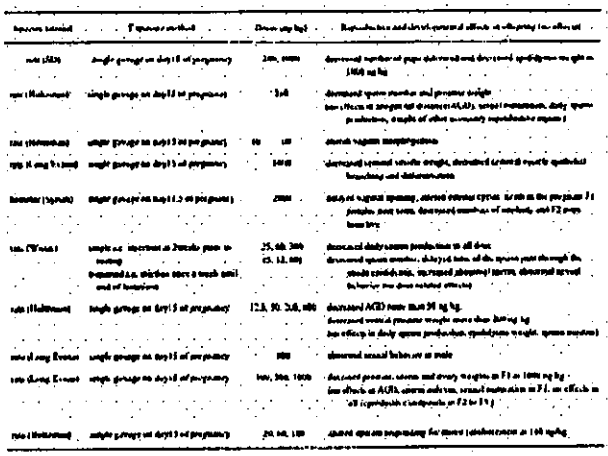
雌Wistarラットに25，60または300 ng/kgのTCDDを交配前2週に皮下投与し，交配前，交配中，妊娠中及び授乳中を通じて，5，12または60 ng/kgのTCDDを週1回皮下投与した[8]．300/60 ng/kg投与群で母ラットの妊娠率が低下し，雄児の血清中テストステロン濃度の低下が認められた．全TCDD投与群の雄児で精巣上体の精子数，精巣の1日精子産生及び精子通過率の低下，異常精子割合の上昇，性行動の異常が観察されたが，1日精子産生以外のエンドポイントに明らかな用量-反応関係はみられなかった．最小毒性量は25/5 ng/kg（0.8 ng/kg/dayに相当）であった．

Holtzmanラットの妊娠15日に12.5，50，200または800 ng/kgのTCDDを単回強制経口投与したときの児に対する影響の検討が国立環境研究所で行われた．50 ng/kgで生後63及び120日における雄児のAGDが短縮し，12.5 ng/kgで生後120日の腹側前立腺重量が減少した[9]．生後2，49または63日に雄児を検査したところ，800 ng/kgでADGの短縮がみられたが，1日精子産生及び精巣上体尾部の精子保有に差は認められなかった[10]．生後49または120日の検査結果では，200 ng/kg以上で腹部前立腺重量の低下が観察され，50 ng/kg以上で生後120日において雄児のAGDが短縮したが，精巣の重量及び組織病理学的検査，1日精子産生，精巣上体重量，精巣上体尾部の精子保有，血清ホルモンレベルにTCDD投与の影響は認められなかった[11]．また，生後49日の雄児の腹側前立腺において200 ng/kg以上で5 α-reductase type 2 mRNAレベルの上昇，50 ng/kg以上でandrogen receptor mRNAレベルの低下が観察された11)．生後5日の雄児の胸腺のCYP1A1 mRNA inductionが200 ng/kg以上でみられている（1群3例の実験であり統計処理については不明）が，胸腺の重量及び細胞数には800 ng/kgでもTCDDの影響は観察されなかった[12]．

Long Evansラットの妊娠15日に800 ng/kgのTCDDを投与したとき，雄児の交尾行動の変化が示されている[13]．また，妊娠15日のLong Evansラットに100，300または1000 ng/kgのTCDDを単回強制経口投与し，F4の離乳まで観察したところ，F1において1000 ng/kgで雄児の前立腺重量の低下及びテストステロンレベルの低下，雌児の子宮及び卵巣重量の低下がみられたが，AGD，精子指標，雌性成熟，繁殖指標にはTCDDの影響は認められず，F2以降に生殖に対する影響は観察されなかった[14]．

妊娠18日に20，60または180 ng/kgのTCDDを単回強制経口投与したHoltzmanラットの雌児における回転かごでの行動変化が180 ng/kgで認められている[15]．

Table 1. Summary of the reproductive and developmental effects by TCDD (published after 1988)



1998年以降の国際機関および各国のダイオキシン評価

1998年WHO-IPCSで行われたダイオキシン類のTDI再評価以後，我が国を始め各国政府及び国際機関でダイオキシン類の健康影響評価と耐容摂取量の勧告状況について以下にまとめた(Table 2)．

1998年のWHO-IPCS専門家会合では，上述したようにTCDDの半減期がヒトとげっ歯類では著しく異なることから，体内負荷量という概念を用いてヒトへの換算を行い，ヒトの体内負荷量から1日摂取量を逆算した後，TDIとして1～4 pg TEQ/kg/dayを勧告した．このとき，算出の基となった最も低い体内負荷量で現れる毒性としては，サルにおける子宮内膜症及び神経行動学的発達への影響とげっ歯類の経胎盤／経母乳暴露による次世代の生殖器官発生異常・免疫抑制であり，最低毒性発現体内負荷量としては28～73 ng/kgと見積もられた．WHO-IPCSではこれらの毒性のうちどれかをTDI算定の根拠とするわけではなく，レンジとして捉え幅のあるTDIを勧告することになった[1]．

我が国では1999年，厚生省と環境庁の合同専門家会合（中央環境審議会環境保健部会，生活環境審議会，食品衛生調査会）において，WHO-IPCS1)の考え方を基本とし，TCDDによる各種毒性影響を体内負荷量の基準とし，結果として数編の経胎盤／経母乳暴露による次世代の生殖器官発生異常・免疫抑制に関する報告をTDIの算定根拠として選択した．その際，最も低い体内負荷量を示したのはFaqiら[6]の報告で27 ng/kg，次にOhsakoら[11]ら（当時は学会発表時のデータを引用した）の43 ng/kgであったが，それらの単報での値を採用するのではなく，実験の信頼と再現性を考慮し，その他の同様の毒性を比較すると，概ね86 ng/kg以上で影響が現れるとすることが妥当であると判断した．また，このときサルの子宮内膜症と神経行動学的発達への影響の実験は，その実験方法に信頼性が担保できないので定量的評価には用いないこととした．その結果，体内負荷量86 ng/kgをTDI算定の出発点とし，不確実係数10を用いて，TDIを4 pgTEQ/kg/dayとした[2]．

米国EPAの2000年の再評価ドラフトでは，ダイオキシン類の最適な毒性指標は発がん性にあるとし，動物実験及びヒトの疫学情報から導き出された体内負荷量をもとに発がん性のリスクを計算した．その結果，1 pgTEQ/kg/dayあたりの発がんリスクは1000分の1であるとし，現在のバックグランドレベルの暴露におけるリスクは100～1000分の1の間にあると算出した．また，本来なら計算されるであろう耐用摂取量に相当するReference doseについては，ヒトのバックグランドレベルを大きく下回ることから算出せず，WHO-IPCSで評価した1～4 pgTEQ/kg/dayというTDIはリスクマネージメントの目的としては妥当であるとしている[10]．

ECのScientific Committee on Food（SCF）が2000年11月に行ったダイオキシン類評価では，WHO-IPCS[1]での評価と同様に，体内負荷量の概念を用い，最低体内負荷量のエンドポイントとして，サルにおける子宮内膜症及び神経行動学的発達への影響とげっ歯類の経胎盤／経母乳暴露による次世代の生殖器官発生異常・免疫抑制を取りあげた．このときの体内負荷量は25～60 ng/kg（WHO-IPCS[1]と同じデータセットを用いているが，主に体内吸収率を60％と少し低めに設定していることや，単回投与の実験結果を亜慢性試験の実験で得られる結果と比較できるように補正しているため異なった値となっている）と見積り，不確実係数9，6からTDIとしては1～3 pgTEQ/kgと計算した．SCFではこのレンジで与えられたTDIの中から単一の値を採用する科学的根拠に乏しいことから，暫定的なTDIとしては，1 pgTEQ/kgにすべきであるとの結論になった．しかし，ダイオキシン類の長い体内残留性を考慮して，1週間単位の耐容摂取量として7 pgTEQ/kg/week（t-TWI：temporary tolerable weekly intake）を勧告することになった[12]．しかし，2001年5月には，新たに公表された報告も加え，暫定値の見直しを行った．新たに，Faqiら[6]とOhsakoら[11]のデータを追加し，最低毒性発現体内負荷量として40～100 ng/kg，無毒性体内負荷量として20 ng/kgをそれぞれ算出した．無毒生体内負荷量からTDIを算出すると，3 pgTEQ/kg/dayとなるが，Faqiら[6]のWistarラットを用いた方が，感受性が高いと考えられ，40 ng/kgからTDIを算出すると2 pgTEQ/kg/dayとなった．前回の評価ではサルの試験も感受性の高いエンドポイントして取りあげられていたが，試験の信頼問題が解決できなかったため，今回は考慮しなかった．さらに，最終的には，前回と同様，長い体内消失半減期を考慮し，1週間耐容摂取量として14 pgTEQ/kg/weekを勧告した[13]．

2001年6月に行われたFAOとWHOの合同食品添加物専門家会議では，EC-SCF[12]での再評価と同様のデータセットを用いて，評価を行ったが，耐容摂取量としては体内中の長い半減期を理由に，ECの評価より長い耐容1ヶ月許容量

（TMI）を勧告した．算定根拠となる体内負荷量としてもSCFの評価と同様に最低毒性発現体内負荷量（Faqiら[8]のデータ）と無毒性体内負荷量（Ohsakoら[11]のデータ）を基に計算を行ったが，その際の慢性投与に対する補正を行った体内負荷量の計算方法は，Linear fit model（直線回帰モデル）とPower fit model（べき乗回帰モデル）という2つの方法を試みている．それらの結果をもとにSCFと同様のTMIを算出すると40～100 pg TEQ/kg/monthの範囲になり，暫定TMIとしては中間値を取って，70 pgTEQ/kg/monthを勧告した[19]．

英国のUK-Food Standards Agency（FSA）ではEC-SCF[18]及びJECFA[19]の考え方を基本的に採用した，最も感受性の高いエンドポイントとして，ラット雄への生殖機能の発生異常（特に精子形成への影響）を用いているが，体内負荷量の算定は，EC-SCF[18]やJECFA[19]とは異なり，最も感受性が高い時期を妊娠16日とし，この時期の胎児の体内負荷量と母体の体内負荷量との比を用いて単回投与と反復投与で得られる体内負荷量の値の補正を行った．その結果，Faqiらのデータに基づいて得られた妊娠16日の母体の体内負荷量は33 ng/kg/dayと見積もられた．この値からEC-SCF及びJECFAと同様の不確実係数（9.6）を用いてTDIとして2 pgTEQ/kg/dayを勧告した．EC-SCF[18]やJECFA[19]では1週間や1ヶ月あたりの耐容量として勧告しているが，TDIとして表現する方が適切で，わかりやすいとする理由で，TDIでの勧告値を採用しているが，仮に短期間でTDIを越える暴露があっても体内負荷量が大きく変動することはなく，長期間にわたった平均値がTDIを下回れば有害影響が現れることはないであろうということが付け加えられている[20]．

Table 2. Summary of tolerable intake derivations for TCDD in the assessment agencies

Assessment agencies	Endpoints in categories	Body burden ng/kg	Accumulated human levels	Uncertainty factor	Tolerable intake
WHO-IPCS	decreased sperm count (rats),		10 pg TEQ/kg		
	immunosuppression (rats),	20 ~ 70	20 pg TEQ/kg	10	2pgTEQ/kg/day
	increased fetal malformations in fetuses (rats),		37 pg TEQ/kg		
	reproductive toxicity in primates (monkeys)		50 pg TEQ/kg		
Japan	decreased sperm count (rats), increased prolactin	50	40	10	4 pgTEQ/kg/day
	postimplantation loss (rats), immune suppression (mice)				
EC-SCF	decreased sperm production (rats)	40		20	2 pgTEQ/kg/day
			20 pg TEQ/kg/day		(1 pgTEQ/kg/day
					33 pg TEQ/kg)
JECFA	decreased sperm production (rats),	20 or 42	420 or 630 pg TEQ/kg/month	9.6	70 pgTEQ/kg/month
	decreased testicular and ovarian weight (mice)	10 or 15	210 or 315 pg TEQ/kg/month	3.2	(60 ~ 90 pg TEQ/kg/month)
U.K.-FSA	decreased sperm production in rats	13	13 pg/kg/day	4.6	2 pgTEQ/kg/day

まとめ

最近のEC，JECFA，UKのダイオキシン類評価では使用されているデータセットは，1999年に我が国で行われた再評価で用いられたものとほぼ同じであるが，Faqiら[8]の1日精子産生低下やOhsakoら[11]の雄児のAGD短縮の報告が最も感受性の高いエンドポイントであるとして体内負荷量を計算し，TDI算定のための出発点として使用している．たとえば，SCF[18]には低い体内負荷量で発現するTCDDの影響として，Holtzmanラットの雄児において64 ng/kg以上の投与量で観察された1日精子産生低下及び精巣上体精子数の減少[21]，Long Evansラットの雄児において50 ng/kg以上の投与量で観察された開眼促進及び射精精子数減少[22]，Wistarラットの雄児において25/5 ng/kg以上の投与量で観察された1日精子産生低下及び性行動の変化[8]，Holtzmanラットの雄児において50 ng/kg以上の投与量で観察されたAGD短縮[11]が記載されている．

Grayら[22]の報告した開眼促進は1日程度の遅れであり，毒性学的意義は弱いと考えられる．また，射精精子数についてはこの実験結果で有意な減少は最高投与量の800 ng/kgだけに認められたものであり，前報[23]の成績と合わせて統計処理すると50 ng/kg以上で有意差が認められたと云う結果である．これらの成績をTDI設定の根拠とすることは不適切と考えられる．

Faqiら[8]の報告した雄児の性行動の変化には用量－反応関係は認められない．

Ohsakoら[9,11]により雄児のAGD短縮が報告されている．AGDの体重による補正値（AGD/cube root of body weight）は示されておらず，AGD短縮の程度は軽度であり，児の生後63日[9]と生後120日[11]に断続的に認められた変化であり，毒性学的な意義は弱いと考えられる．

TCDDの精子細胞，精子に対する影響については多くの報告があるが，TCDDの精子細胞，精子に対するTCDDの影響には報告間で差がみられる．Ohsakoら[9,10,11]は精子指標及び精巣の病理組織学的所見には800 ng/kgまでのTCDD投与の影響は認められなかったとしている．米元ら[14]は1000 ng/kgのTCDD投与でも精子検査においてTCDDの影響は認められず，同様なプロトコールによるHoltzmannラット[21,24,25]及びLong Evansラット[22,26]を用いて行われた実験結果を再現できなかったと述べている．Grayら[22]は精巣の精子産生はTCDDの鋭敏な指標ではないと述べており，また，TheobaldとPeterson[27]はラット，ハムスター，マウスの3種の動物で観察される唯一の生殖発生毒性のエンドポイントは精巣上体の精子数減少であると述べている．このように，低用量のTCDDの精子指標（1日精子産生，精巣上体精子数）に対する影響については実験間で整合性のある結果は得られていない．

一方，JECFA[19]の評価でもFaqiら[8]やOhsakoら[11]の両報告を用いて様々な体内負荷量の計算を試みているが，結局，どちらか単一の報告を基にするのではなく，両報告から得られた体内負荷量のレンジの中央値をTDI算定の出発点としたことからも，この両報告に対する毒性学的意義付けは必ずしも確定していないと考えられる．また，Rierら[28]およびScantz & Bowman[29]のアカゲザルにたいする影響も，1999年以後，未だにその実験の信頼性に関する問題は依然解決されていなく，EC，JECFA，UKにおける耐容摂取量

算定のための定量的評価にも実質的には使用されていない．

以上のことから，TDI算定の出発点となる最低の毒性発現体内負荷量の算定のためにFaqiら[8]やOhsakoら[11]の両報告結果を用いる積極的な理由は現在のところなく，1999年の我が国のTDI再評価に用いたGrayら[22,25]およびGehrsら[30]の報告で母動物にTCDDを投与した場合に児動物に見られる開眼促進，精巣上体精子数の減少，雌性生殖器形態異常，遅延型過敏症の抑制最低の毒性発現体内負荷量：86ng/kgを出発点としてTDIを算定することは現在でも妥当であると考えられる．

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Workshop 6.2

Hormonally active agents and plausible relationships to adverse effects on human health*

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Abstract: A hormonally active compound was first identified in the book *Silent Spring* by Rachel Carson in 1962, implicating the effect of pesticides such as DDT and the derivatives. Nearly four decades later, the book *Our Stolen Future* by Theo Colborn et al., and other pertinent publications have revisited and broadened the issue regarding a variety of possible chemicals and the area exposed. Translation and publication became available in Japan within the last four years. Since then, Japan joined the member countries involved in the global issue of endocrine disruptors, the "environmental hormone".

Although a significant number of chemicals possessing a hormone-like action have been recognized for many years, and the action of their biological plausibility related to the receptor-mediated effects strongly suggests possible human effects comparable to hormonal changes in wildlife, little is known about evidences or adversities in experimental animals and humans. The most essential key to resolving these dilemmas may be to understand the mechanism of actions (i.e., a possible low-dose issue). In other words, the mechanism at the low-dose effect may be resolved simultaneously by the mechanism of three major questions linked to the low-dose issue; namely, threshold, possible oscillation, and additive and/or synergistic action.

INTRODUCTION

The objective of this paper is to summarize all currently available information on hormonally active agents and plausible relationships to adverse effects on human health from the standpoints of the mechanisms of action of these chemicals.

It is not uncommon to come across agrochemicals and industrial chemicals that have hormone-mimic effects. These chemicals, the so-called "environmental hormones", often accumulate at detectable levels in the environment, and it has been feared that they may have adverse effects not only on wildlife but also on human beings. Following reports of feminization and decreased colony size of wild creatures, and reports suggesting a possible association of these chemicals with abnormalities of reproductive organs and oncogenesis in humans, attention has focused on the possibility that these occurrences may be associated with exposure to endocrine-disrupting chemicals (EDCs). In this connection, we would like to draw the attention of the reader to a Japanese translation of the book *Our Stolen Future*, written by Theo Colborn et al.

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This paper will review the subjects related to EDCs, the courses of arguments regarding the possible hazards of these chemicals, and current medical subjects pertaining to them.

CHEMICALS WITH HORMONE-MIMIC ACTIONS

Substances with hormone-mimic effects can be divided into four groups:

- hormones found in vivo;
- medicines with hormone-mimic actions manufactured for use in hormonal therapy, etc.;
- plant hormones known to exert phytoestrogen-like actions; and
- chemicals found in environments that can interact with hormone receptors.

In addition, substances that do not interact with hormone receptors but exert effects on gonads by their modifying effects on steroid metabolism may be deemed as hormone-mimics in the broader sense of the term. In this paper, however, emphasis shall be placed on the hormone-mimic actions mediated by receptors that play essential roles in the mechanism of actions of hormone-mimics.

CHARACTERISTICS OF THE RECEPTOR-MEDIATED ACTIONS OF HORMONE-MIMICS

The receptor-mediated actions of hormone-mimics are fundamentally characterized by the similarity in structures of the receptors involved, crossing the barrier of animal species. These characteristics allow us to speculate the possibility that the actions of these chemicals exerted in nature may also occur in humans.

Second, since similarities in the structure of various sex steroids and hormones are also known, it is possible that each individual hormone-mimic exerts diverse effects by acting on male hormone receptors, female hormone receptors, and nuclear receptors (including many orphan receptors), etc.

Third, many of these chemicals are excreted from the living body in the form of conjugated inactive substances instead of as degraded metabolites. They may also be eliminated in the unchanged form. Therefore, if feces and urine containing these substances are eliminated into river water, it is plausible to imagine that even inactivated hormones can sometimes become active and exert hormone-mimic actions in the environment. This is one of the characteristics unique to this class of chemicals.

Receptor-mediated responses involve many unresolved questions. Various undefined elements may be involved, including the relationship between receptor binding and signals, the relationship between receptor-ligand binding (ligand: substances that can bind to receptors) and the dissociation of ligands from receptors, signal cross-talks, involvement of unknown nuclear receptors, etc.

The actions of these chemicals add to the effects of intrinsic hormones. For this reason, these chemicals may exert their actions in a way different from that known for other chemicals that do not have structural or functional counterparts in vivo. For example, stimulation of hormone receptors by these extrinsic chemicals may modify homeostasis in vivo, leading to down-modulation of the physiological stimulation of these receptors by the intrinsic ligands. Therefore, the influence of the continued effects of environmental hormones needs special study.

PITFALL IN THE EFFECTS OF HORMONE-MIMICS

We must distinguish the interactions of endocrine hormone-mimics with hormone receptors from the hazards caused to endocrine tissue. Bearing this in mind, let us now summarize the problems related to the effects of hormone-mimics.

Antagonistic effects maintaining homeostasis

The endocrine system is regulated by homeostatic mechanisms. It is not uncommon for the effects of small amounts of hormone-mimics to interfere slightly with these mechanisms, often with no adverse influence; this is well known. However, this is not always the case. There seems to be a group of genes that act antagonistically to each other in the maintenance of homeostasis.

With the uterotrophic assay, which is used to check for estrogenic activity, the ovary is removed in advance and the blood level of the intrinsic female hormone is reduced to the minimum. Under the thus-created extremely shrinking state of the uterus, the test substance (a chemical or hormone) is administered to evaluate for its effects on the inflation of the uterus. This test (checking for growth of the uterus in ovariectomized animals) is designed to evaluate the hormone activity and effects of hormone-mimics under conditions of blockade of homeostasis.

This test method itself is valid. However, there is no sufficient rational evidence that indicates that the responses observed under such indirect control conditions of the living body can serve as an indicator of the health hazards of hormone-mimics. Although the ovo-testes seen in lower vertebrates may be used if the effects observed were to be valid as such an indicator, there is no consensus on what is valid as an indicator of the health hazards of EDCs when mammals are used as experimental animals.

Down-regulation of the expression of receptors

It is known that the expression of gene-encoding receptors is down-regulated by continuous stimuli, leading to reduced receptor activity. This can lead to a paradoxical outcome wherein the effects observed in the presence of low levels of a substance are not seen at high levels of the same substance. If this phenomenon occurred in individual organisms, the dose–response relationship will be nonlinear.

This means that extrapolation of results obtained at high levels of the chemicals, to conditions where low levels of the same substance are present, would be difficult. It is needed to test the validity of this hypothesis; analysis of the mechanisms underlying this phenomenon if the hypothesis were indeed valid, are thus important. Studies to resolve these questions are now under way.

Data gap concerning the effects of female hormones

In mature women, there are high levels of physiological hormones in vivo, and these are subject to cyclic control. It has been proposed that girls with inadequate physical growth begin menstruation at lower ages and undergo sexual maturation earlier than usual, and that hormone-mimics in these subjects can precipitate breast cancer.

The weak links in this hypothesis have been pointed out, and it has been shown experimentally that estrogen by itself may be teratogenic, although this tendency has been shown to be weak. It is known that organisms are programmed such that excessive exposure to estrogens during the intrauterine period or other developmental stages is avoided.

There are many open questions as to the mechanism by which mature females remain physiologically stable, even when exposed daily to high levels of estrogen (400 pM/l). Some additional dramatic effects may be needed to disturb this homeostatic physiology.

Multigeneration tests and effects on fetuses

It has been shown that exposure to hormones or hormone-mimics during intrauterine or early neonatal periods can lead to irreversible changes in the pattern of development. This susceptibility period is short, extending from the 13th gestational day to about one week after birth. These effects are the so-called "intrauterine window effects."

In animal studies involving observation of experimental animals for two or more generations, no effects of EDCs have been demonstrated. The question therefore arises as to why window effects are observed during the short period mentioned above. It is unknown whether or not these effects really do occur, and if they do, how they are produced.

Delayed growth of the thalamic nucleus specific to males (called sexual dimorphic nucleus) is seen in male rats treated with female hormones. We may say that under conditions of homeostasis of the physiological hormones in mature individuals, exposure to dose levels that usually cause only reversible changes can lead to irreversible changes, if the exposure occurs during genesis, morphogenesis, or functional development. However, there are no ample data endorsing this view in humans.

Considering the biological plausibility inferred from the experimental data accumulated to date*, we may say that there are no sufficient data that clearly rule out this view. Close attention has therefore been paid to these effects in children.

New theories of methodology, focusing on effects in fetuses and children, are now being developed, primarily in the United States, or the World Health Organization, within the framework of children's program, etc.

HEALTH HAZARDS AT LOW LEVELS OF EXPOSURE

Chemicals used for agriculture or industrial purposes are marketed, in general, only after their effects on living beings have been investigated. We may therefore understand that they are used on the premise that the possibility of these chemicals exerting hazardous effects on health at relatively high-dose levels has been almost ruled out. Nevertheless, problems with EDCs have begun to be highlighted. These problems may not be confined to those related to the accumulation of these substances through food chains in the ecosystem, but also to the additional possibility that these chemicals may exert effects at low-dose levels even if they have been declared safe at high-dose levels. The latter possibility may apply, however, only to some cases and not to others.

We may say that a major issue pertaining to EDCs that must be resolved urgently is whether or not they pose health hazards at low-dose levels. This issue can be summarized into the following three questions:

- presence/absence of threshold level;
- presence/absence of synergistic or additive effects; and
- possibility of extrapolation of high-dose effects to low-dose levels (i.e., presence/absence of a linear dose–response relationship).

No clear-cut answers have as yet emerged to these questions. Considering the above-mentioned characteristics of the effects of hormones, it is plausible to imagine how difficult it may be to resolve these questions.

To determine if these chemicals exerted hazardous effects on health at low-dose levels, the following basic questions may need to be considered; their biological plausibility is hardly denied.

- Regarding the presence or absence of threshold levels, it seems likely that many chemicals suspected of being EDCs can easily permeate across the cell membrane, which is composed of phospholipids. Therefore, assuming that one receptor molecule reacts with one chemical molecule, the lower limit of the dose level exerting the chemical's effects would be extremely low.

  Of course, since the probability of the binding of a ligand to the receptor will be low if the dose level is low, we cannot say that there is no threshold level for the effects seen in the low-

*Biological plausibility: Likelihood of a phenomenon as judged by considering the difference or similarity of elements of reactions in individual organisms, on the basis of the results of a series of related biological experiments. (Cf. probability)

dose-level range. In fact, for bisphenol A (which has been attracting close attention because of its hazardous effects on health at low-dose levels), the presence/absence of a threshold level has not yet been reported. It seems rational, therefore, to assume that these health hazards occur in a very low-dose-level range.

- If we consider not only the affinity of each substance for the receptor, but also the nonlinearity of responses (e.g., waveform responses as a result of reduced receptor expression following an increase in dose level), it is possible to assume that there are U-shaped or reverse U-shaped reactions, or oscillational dose–response curves. Interim data endorsing such a view are being accumulated.
- Regarding the possibility of synergistic or additive effects, the observation of additive effects among different nuclear receptors has been reported. Data yielded by analysis of interactions between receptor signals also suggest such a possibility. In fact, the dose–response curves for some composite materials were reported to be additive, but not synergistic.

Thus, the questions on health hazards at low-dose levels have several aspects:

- type of receptor-mediated actions of the hormone mimics;
- diverse reactive characteristics on the part of the receptors;
- diverse modification during expression of intracellular signals; and
- factors involved in irreversible changes related to morphogenesis and functional development.

Resolution of all these aspects of the question will lead to clarification of the mechanism of actions of the substances from each of the aforementioned standpoints. While these questions are among the hottest research themes at present, they are certainly unlikely to be resolved easily.

At a workshop held in North Carolina, USA, in October 2000, health hazards of chemicals at low-dose levels were discussed. Investigators for and against the possibility of these substances posing health hazards at low-dose levels gave detailed accounts of their studies, and no definitive conclusions could be reached, as the arguments of both sides appeared to be tenable.

This means that reports affirming the plausibility of these substances posing health hazards at low-dose levels in animal experiments cannot be immediately rejected. The workshop concluded by pointing out the necessity of paying attention to the possible hazards on fetuses and neonates.

HEALTH HAZARDS OF HORMONE-MIMICS TO HUMANS

The possibility of health hazards of hormone-mimics to human beings have not been supported by adequate epidemiological data, and the number of cases for which the data clearly endorse such effects is quite small. The U.S. National Research Council (NRC) emphasizes the necessity of conducting further epidemiological studies on this topic (NRC, 1999).

In conclusion, this paper summarizes the current knowledge concerning the health hazards of hormone-mimics to humans. Reports dealing with the effects of these substances on humans are confined to those pertaining to the effects of dioxins and polychlorinated biphenyls (PCBs); the validity and usefulness of these results have not yet been established.

The following information is based on case studies conducted to date.

Health hazards of dioxins

Regarding health hazards of dioxins, two-year dosing studies revealed weight loss and liver damage, and three-generation reproductive studies in rats disclosed intrauterine death and a decrease in litter size. Onset of endometriosis in rhesus monkeys has also been reported.

A causal relationship of EDCs to the following episodes in humans has been suggested: biased male-to-female ratio in children born in the dioxin-exposed Seveso area of Italy, and increased inci-

dence of cleft palate in the Diemerzeedijk district of the Netherlands, probably due to steroids. In both of these cases, the U.S. Environmental Protection Agency (USEPA) did not affirm a causal relationship, and classified them as cases requiring special attention.

No consensus has been reached concerning the relationship of hypothyroidism observed in the inhabitants along Lake Michigan to the ingestion of PBB- (polybrominated biphenyls-) contaminated fish.

Effects on mature females (e.g., increased incidence of breast cancer)

No reports affirm the effects of dioxins on mature human females (e.g., effects on breast cancer or endometriosis as discussed below). There are many unresolved questions on this topic. However, none of the studies conducted in mature experimental animals revealed data endorsing the plausibility of occurrence of such effects. On the other hand, it is known that the age at menarche is lower and the incidence of breast cancer higher in females exposed to dioxins. Some investigators cite these data when discussing the health hazards of dioxins. It is also known that females exposed to dioxins are often taller.

In European countries, a height increase of about 3.5 mm per year and an approximately one-year decrease in the age at menarche have been reported during the past 30 years. It is difficult to identify the influence of extrinsic endocrine factors on these changes, and no studies addressing this issue have been reported to date. Although several studies have been published concerning the effects of female hormone preparations, including pills used for contraception and hormone replacement therapy in postmenopausal women, no studies have provided data that establish the effects of EDCs.

Endometriosis

Endometriosis is a disease of unexplained origin that is seen in primates with sexual cycles. It has been pointed out that this disease tends to be more severe in individuals exposed to dioxins (2,3,7,8-tetrachlorodibenzo-*p*-dioxin [TCDD] and to PCBs). Data yielded from experiments in rhesus monkeys are used as evidence to corroborate the causal relationship between dioxins and endometriosis. Thus, we cannot rule out the biological plausibility of these effects. However, no reports affirming the causal relationship in humans have been published.

Possibility of other effects on humans

Biological plausibility has also been considered for the following effects of hormone-mimics on humans: qualitative dysfunction of human sperm, effects on neurobehavior of neonates, and immune functions. The effects on immune functions have been suggested by reports of cases with Yu-sho (PCB intoxication).

CONCLUSION

The International Program of Chemical Safety (IPCS), a section of the World Health Organization, has released a Web site publication "Global Assessment of the State-of-the Science of Endocrine Disruptors" (GAED), June 2002 (URL: <http://ehp.niehs.hih.gov/who/>). WHO/IPCS started the GAED program in March 1998 after the publication of *Our Stolen Future* (Theo Colbone et al., 1996). The publication took three years to edit; covering a policy to document all the published pertinent literatures, to summarize them as descriptive manner solely based on those published literatures. Twenty-seven expert scientists and 20 independent peer-reviewers participated in editing the GAED.

Other reports on nonylphenol and octylphenol, released by the Japanese Ministry of Environment (MoE), revealed an "ovotestes" formation that was observed in the assay of the laboratory experimen-

tal fish (*Medaka*) exposed to doses close to those recorded in the monitoring fields in the MoE surveillance. Further, phthalates, such as di-(2-ethylhexyl)phthalate, di-cyclohexylphthalate, and butylbenzylphthalate, as selected and prioritized chemicals by the MoE, showed some unique data in different endpoints, including mRNA expression, in dose ranges lower than those no observed effect levels (NOELs) and/or no observed adverse effect levels (NOAELs) reported previously.

The effects of EDCs on human health are unknown at this moment. However, due to the biologically plausible data currently accumulated, the existence of endocrine disruptions under certain circumstances seems to be a reality. Thus, by the time of the SCOPE/IUPAC symposium, the EDC research for the next stage may shift from plausibility to possibility, and put forward further mechanistic research.

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Implications of Endocrine Active Substances for Humans and Wildlife: a SCOPE/IUPAC Project

- Molecular Mode of Action of Nuclear Receptor Fundamentals for Understanding the Action of Endocrine Active Substances
- Environmental Fate and Metabolism of Endocrine Active Substances
- Effects of Endocrine Active Substances in Rodents and Humans and Their Implications for Humans
- Effects of Endocrine Active Substances in Wildlife Species
- Effectiveness of Tests for Characterizing of Endocrine Disruptor Hazard
- Considerations on a Rational Approach to Endocrine Disruptor Research
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- Recommendations Concerning Validation and Weight of Evidence in Endocrine Disruptor Issues
- Risk Management Options for Endocrine Disruptors in National and International Programs

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