mechanism by which mature females remain physiologically stable, even when exposed daily to high levels of estrogen (400pM/*l*). Some dramatic effects are probably needed to disturb this physiology.

4. Multi-generation 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 EDs 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 are they produced.

Delayed growth of the thalamic nucleus specific to males (called sexual type II 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,[*1] 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 the effects in fetuses and children, are now being developed, primarily in the United States, 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 EDs have begun to be highlighted. These problems may be not confined to those related to the accumulation of these substances through food chains in the ecosystem, but also to the possibility additionally 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 EDs 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: (1) presence/absence of threshold level, (2) presence/absence of synergistic or additive effects, and (3) 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.

[*1] 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 a related biological experiments. (cf. probability)

(1) Regarding the presence or absence of threshold levels, it seems likely that many chemicals suspected of being EDs 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 very 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 dose level range. In fact, for bisfenol 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.

(2) If we consider not only the affinity of each substance for the receptor, but also the non-linearity 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.

(3) 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: (1) the type of receptor-mediated actions of the hormone mimics, (2) diverse reactive characteristics on the part of the receptors, (3) diverse modification during expression of intracellular signals, and (4) 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 on Humans

The possibility of health hazards of hormone-mimics on humans 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 US National Research Council emphasizes the necessity of conducting further epidemiological studies on this topic (National Research Council, 1999).

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

The following are based on case studies conducted to date.

1. 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 EDs 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 incidence of cleft palate in the Diemerzeedijk district of the Netherlands, probably due to steroids. In both of these cases, the Environmental Protection Agency (EPA) of the United States did not affirm a causal relationship, and treated 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.

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

No reports affirming 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 a number of 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 EDs.

3. 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 (TCDD/PCBs). Data yielded from experiments in rhesus monkeys are used as evidence to corroborate the causal relationship between dioxins and endometriosis. We cannot thus rule out the biological plausibility of these effects. However, no reports affirming the causal relationship in humans have been published.

4. Possibility of other effects on humans

Biological plausibility has been pointed out also on 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).

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Evaluation of developmental toxicity of 1-butanol given to rats in drinking water throughout pregnancy

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Abstract

The objective of this study was to evaluate the developmental toxicity of 1-butanol in rats. Pregnant rats were given drinking water containing 1-butanol at 0.2%, 1.0% or 5.0% (316, 1454 or 5654 mg/kg/day) on days 0–20 of pregnancy. A significant decrease in maternal body weight gain accompanied by reduced food and water consumption was found at 5.0%. No significant increase in the incidence of pre- and postimplantation embryonic loss was observed in any groups treated with 1-butanol. Fetal weight was significantly lowered at 5.0%. Although a significant increase in the incidence of fetuses with skeletal variations and decreased degree of ossification was found at 5.0%, no increase in the incidence of fetuses with external, skeletal and internal abnormalities was detected in any groups treated with 1-butanol. The data demonstrate that 1-butanol is developmental toxic only at maternal toxic doses. No evidence for teratogenicity of 1-butanol was noted in rats. Based on the significant decreases in maternal body weight gain and fetal weight, it is concluded that the no observed adverse effect levels (NOAELs) of 1-butanol for both dams and fetuses are 1.0% (1454 mg/kg/day) in rats.

Keywords: 1-Butanol; Developmental toxicity; Teratogenicity; Fetal abnormality; Rat

1. Introduction

1-Butanol (CAS no. 71-36-3, *n*-butanol; *n*-butyl alcohol), a flammable colorless liquid with a rancid sweet odor, is widely used as an organic solvent and intermediate in the manufacture of other organic chemicals (IPCS/WHO, 1987). Exposure of the general population is mainly through its natural occurrence in food and beverages and its use as a flavoring agent (IPCS/WHO, 1987).

Several reports on the developmental toxicity of 1-butanol are available. Nelson et al. (1989a) reported the results of a developmental toxicity study in which SD rats were exposed to 1-butanol by inhalation for 7 hr/day on days 1–19 of pregnancy at 3500, 6000 and 8000 ppm (equivalent to estimated daily absorbed doses of 350, 600 and 800 mg/kg). They observed maternal deaths at 8000 ppm, decreases in maternal food consumption and fetal weight at 6000 and 8000 ppm, and an increased incidence of rudimentary cervical ribs at 8000 ppm, and concluded that 1-butanol was not a selective developmental toxicant in rats. Nelson et al. (1989b) conducted a behavioral teratology study in which female SD rats were given 1-butanol by inhalation at 3000 or 6000 ppm for 7 hr/day throughout pregnancy (the maternal exposure group); male rats were

Abbreviations: NOAEL, no observed adverse effect level

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similarly exposed for 6 weeks and mated to unexposed females (the paternal exposure group), and offspring were behaviorally and neurochemically examined. The data from all tests in their study were within the range of control data in other research conducted by their laboratory. Sitarek et al. (1994) reported a significant increase in the incidence of fetuses with abnormalities after administration of 1-butanol at 0.24–4.0% (300–5000 mg/kg/day) in drinking water during the pre-mating period for 8 weeks and throughout the mating and pregnant period. No maternal toxicity was found at any dose of 1-butanol. The no observed adverse effect level (NOAEL) was not derived from the results of their study, because significant increases in the incidence of fetuses with dilation of the subarachnoid space and dilation of the lateral ventricle and/or third ventricle of the brain were found even at the lowest dose (0.24%). They have concluded that 1-butanol is a developmental toxicant and produces anomalies in the skeleton and central nervous system.

The present study was conducted to determine whether or not morphological abnormalities could be produced in fetuses of rats given 1-butanol prenatally and designed to replicate the observations of the study by Sitarek et al. (1994).

2. Materials and methods

This study was performed in compliance with regulatory guidelines (MHW, 1997a) and accordance with the principles for Good Laboratory Practice (MHW, 1997b) and "Guidance for Animal Care and Use" of Ina Research, Inc.

2.1. Animals

International Genetic Standard (Crj: CD (SD) IGS) rats were used throughout this study. This strain was chosen because it is most commonly used in reproductive and developmental toxicity studies and historical control data are available. Males at 10 weeks of age and females at 9 weeks of age were purchased from Tsukuba Breeding Center, Charles River Japan, Inc., (Yokohama, Japan). The rats were acclimated to the laboratory for 7 days prior to the start of the experiment. Male and female rats found to be in good health were selected for use. Animals were reared on a basal diet (NMF; Oriental Yeast Co., Ltd., Tokyo, Japan) and water ad libitum and maintained in an air-conditioned room at 21–25 °C, with a relative humidity of 40–70%, a 12-h light/dark cycle, and ventilation with 16 air charges/hour. Virgin female rats were mated overnight with male rats. The day when sperm were detected in the vaginal smear was considered to be day 0 of pregnancy. The pregnant rats, weighing 217–273 g and 10–11 weeks of age, were distributed using a computerized randomization procedure (TOXstaff 21 system) into 4 groups of 20 rats each and housed individually.

2.2. Chemicals and dosing

1-Butanol was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The 1-butanol used in this study was 99.9% pure and a special grade reagent (Lot no. CER5688), and it was kept in a dark place at room temperature under airtight conditions. The purity and stability of the chemical were verified by analysis before and after the study. Rats were given 1-butanol in their drinking water at a concentration of 0 (control), 0.2%, 1.0% or 5.0% on day 0 through day 20 of pregnancy. The dosage levels were determined based on the results of our range-finding study in which administration of 1-butanol in the drinking water on days 0–20 of pregnancy caused decreases in maternal body weight gain and food and water consumption and tended to reduce in fetal weight at 4% and 7% in rats. 1-Butanol was dissolved in distilled water (Otsuka Pharmaceutical Factory, Inc., Naruto, Japan). The control rats were given only water. The stability of formulations in a dark and cool place under airtight conditions has been confirmed for up to 3 days. During use, the formulations were maintained under such conditions for no more than 3 days and were 95.7–103.5% of the target concentration.

2.3. Observations

The maternal body weight and water consumption were recorded daily, and food consumption was recorded every 3 or 4 days. The pregnant rats were euthanized by exsanguinations under ether anesthesia on day 20 of pregnancy. The peritoneal cavity was opened, and the numbers of corpora lutea, implantation sites and live and dead fetuses and resorptions were counted. The live fetuses removed from the uterus were sexed, weighed, measured among their crown-rump length, and inspected for external malformations and malformations within the oral cavity. Approximately one-half of the live fetuses in each litter were randomly selected and fixed in alcohol, stained with alizarin red S (Dawson, 1926) and examined for skeletal anomalies. The remaining live fetuses in each litter were fixed in Bouin's solution. Their heads were subjected to a free-hand razor-blade sectioning (Wilson, 1973) and the thoracic areas were subjected to microdissecting (Nishimura, 1974) to reveal internal abnormalities. The placental weight was also measured.

2.4. Data analysis

The statistical analysis of fetuses was carried out using the litter as the experimental unit. The initial body

weight, body weight gain and food and water consumption of pregnant rats, numbers of corpora lutea, implantations and live fetuses per litter, fetal weight and crown-rump length and placental weight were analyzed with Bartlett's test (Snedecor and Cochran, 1980) for homogeneity of variance at the 5% level of significance. If it was homogeneous, the data were analyzed using Dunnett's multiple comparison test (Dunnett, 1955) to compare the mean of the control group with that of each dosage group, and if it was not homogeneous, the mean rank of the 1-butanol-treated groups was compared with that of the control group with the Dunnett type test. The Dunnett type test was used for the incidences of pre- and postimplantation embryonic loss and fetal anomalies and sex ratio of fetuses to compare the mean rank of groups treated with 1-butanol and that of the control group. The incidence of dams with anomalous fetuses was analyzed by Chi-square test or Fisher's exact test. The significance of differences from the control group was estimated at probability levels of 1% and 5%.

3. Results

Table 1 shows the maternal findings in rats given 1-butanol during pregnancy. No death was found in female rats of any group. All females in all groups became pregnant. The body weight gains on days 0–7 of pregnancy were significantly reduced at 5.0%. The body weight gain during the whole period of pregnancy was also significantly decreased at 5.0%. No significant decrease in the body weight gain was noted at 0.2 or 1.0, except for a transient decrease on days 0–2 of pregnancy at 1.0%. The food consumption on days 0–7, days 7–14, days 14–20 and days 0–20 of pregnancy was significantly lower in the 1.0% and 5.0% groups than the control group. The water consumption on days 0–7 at 1.0 and 5.0% and on days 7–14, days 14–20 and days 0–20 at 5.0% was significantly decreased. The mean daily intakes of 1-butanol were 316 mg/kg for the 0.2% group, 1454 mg/kg for the 1.0% group and 5654 mg/kg for the 5.0% group.

Reproductive findings in rats given 1-butanol during pregnancy are presented in Table 2. No litters totally resorbed were found in any group. No effects of the administration of 1-butanol were observed on the numbers of corpora lutea, implantations, pre- or postimplantation loss, resorptions or dead or live fetuses or sex ratio of live fetuses. The body weights of male and female fetuses were significantly lower in the 5.0% group than in the control group. There was no significant difference in the crown-rump length of male and female fetuses or placental weight between the control and groups treated with 1-butanol.

A summary of morphological findings in live fetuses of rats given 1-butanol during pregnancy is shown in Table 3. One fetus with spina bifida in the control group and one fetus with thread-like tail and anal atresia in the 0.2% group were observed. Skeletal examination

Table 1
Maternal findings in rats given 1-butanol on days 0–20 of pregnancy

Dose (%)	0 (Control)	0.2	1.0	5.0
No. of rats	20	20	20	20
No. of pregnant rats	20	20	20	20
No. of dead rats	0	0	0	0
Initial body weight	245 ± 14	247 ± 13	245 ± 11	244 ± 12
Body weight gain during pregnancy (g)[a]				
Days 0–7	44 ± 7	45 ± 7	40 ± 6	20 ± 28**
Days 7–14	40 ± 6	41 ± 5	41 ± 7	42 ± 10
Days 14–20	78 ± 14	82 ± 8	84 ± 7	75 ± 11
Days 0–20	162 ± 19	168 ± 16	165 ± 15	146 ± 16**
Food consumption during pregnancy (g)[a]				
Days 0–7	179 ± 12	180 ± 16	164 ± 12*	138 ± 21**
Days 7–14	193 ± 14	194 ± 17	177 ± 14**	160 ± 11**
Days 14–20	176 ± 14	175 ± 15	161 ± 12**	143 ± 11**
Days 0–20	548 ± 38	548 ± 46	503 ± 34**	441 ± 34**
Water consumption during pregnancy (ml)[a]				
Days 0–7	284 ± 28	305 ± 37	258 ± 29*	175 ± 34**
Days 7–14	318 ± 35	337 ± 48	299 ± 40	239 ± 80**
Days 14–20	328 ± 47	342 ± 47	334 ± 46	256 ± 85**
Days 0–20	930 ± 105	983 ± 126	890 ± 106	669 ± 182**
Mean daily intakes of 1-butanol (mg/kg)[a]	0	316 ± 30	1454 ± 186	5654 ± 1402

*,** Significantly different from the control, $*P < 0.05$ and $**P < 0.01$.
[a] Values are given as the mean ± SD.

Table 2
Reproductive findings in rats given 1-butanol on days 0–20 of pregnancy

Dose (%)	0 (Control)	0.2	1.0	5.0
No. of litters	20	20	20	20
No. of litters totally resorbed	0	0	0	0
No. of corpora lutea per litter[a]	16.4 ± 3.6	16.7 ± 3.0[d]	16.1 ± 2.1	16.3 ± 2.6
No. of implantations per litter[a]	14.3 ± 2.8	15.1 ± 1.7	15.2 ± 1.2	14.7 ± 2.5
% Preimplantation loss per litter[b]	9.0	9.0[d]	4.4	9.2
% Postimplantation loss per litter[c]	6.0	5.4	3.7	8.0
No. of live fetuses per litter[a]	13.4 ± 2.6	14.3 ± 1.4	14.7 ± 1.5	13.5 ± 2.5
Sex ratio of live fetuses (male/female)	128/139	145/140	149/144	131/139
Body weight of live fetuses (g)[a]				
Male	4.18 ± 0.27	4.00 ± 0.24	4.04 ± 0.25	3.83 ± 0.18**
Female	3.97 ± 0.25	3.86 ± 0.20	3.83 ± 0.16	3.59 ± 0.17**
Fetal crown-rump length (mm)[a]				
Male	40.5 ± 1.2	40.3 ± 1.4	40.2 ± 1.2	39.7 ± 1.3
Female	39.4 ± 1.2	39.4 ± 1.2	39.3 ± 1.1	38.5 ± 1.4
Placental weight (g)				
Male	0.50 ± 0.05	0.49 ± 0.05	0.48 ± 0.06	0.50 ± 0.06
Female	0.49 ± 0.05	0.48 ± 0.05	0.47 ± 0.05	0.49 ± 0.06

** Significantly different from the control, $P < 0.01$.
[a] Values are given as the mean ± SD.
[b] (No. of preimplantation embryonic loss/no. of corpora lutea) × 100.
[c] (No. of resorptions and dead fetuses/no. implantations) × 100.
[d] Value was obtained from 19 pregnant rats.

revealed one fetus with supernumerary thoracic vertebral bodies and malpositioned thoracic vertebrae at 1.0%. Although the total number of fetuses with skeletal variations was significantly increased at 5.0%, the number of fetuses with individual skeletal variations was not significantly increased, except for fetuses with short supernumerary ribs at 5.0%. A significantly lower number of forepaw proximal phalanges was observed at 5.0%. Membranous ventricular septum defect occurred in one fetus of the control and 0.2% groups and 3 fetuses in 3 dams of the 5.0% group. One fetus with a double aorta in the control group and one fetus with a left umbilical artery in the control and 2.0% groups were observed. Thymic remnants in the neck were found in 4–11 fetuses of the control and groups treated with 1-butanol. However, there was no significant difference in the incidence of fetuses with internal abnormalities between the control and groups treated with 1-butanol.

4. Discussion

The present study was conducted to determine the developmental toxicity of 1-butanol and designed to replicate the observations of the study by Sitarek et al. (1994). The data showed that prenatal administration of 1-butanol did not produce morphological anomalies in fetuses of rats. Thus, we have been unable to confirm the results of Sitarek's study in which prenatal exposure to 1-butanol produced fetal anomalies.

The doses of 1-butanol used in the present study expected to induce maternal and/or developmental toxicity, such as a decrease in maternal body weight gain and fetal weight, were given to pregnant rats during the whole period of pregnancy to characterize the effects of 1-butanol on embryonic/fetal development. Maternal toxicity, a significant decrease in body weight gain, was found at 5.0%. Maternal food and water consumptions were also reduced in this dose group. Although the only significant decrease in maternal body weight gain was observed on days 0–2 of pregnancy at 1.0%, this decrease was occasional and discontinuous and seems unlikely to be of toxicological significance. In this dose group, decreases in the maternal food consumption during the whole period of pregnancy and water consumption during the early period of pregnancy, which were unaccompanied by the continuous changes in body weight gain, were observed. No significant changes in maternal parameters were noted in the 0.2% group. These findings in maternal rats indicate that 1-butanol exerts maternal toxicity at 5.0% (equivalent to 5654 mg/kg/day) when administered during the entire period of pregnancy in rats.

No significant increase in the incidence of postimplantation loss was found at any dose of 1-butanol, and significantly decreased weights of male and female fetuses were found at 5.0%. No significant adverse effects on reproductive parameters were detected at 0.2% and 1.0%. These findings indicate that 1-butanol is not toxic to embryonic/fetal survival up to 5.0% or fetal growth up to 1.0% when administered during the whole period of pregnancy.

As for morphological examinations in the fetuses of exposed mothers, a few fetuses with external, skeletal

Table 3
Morphological examinations in fetuses of rats given 1-butanol on days 0–20 of pregnancy

Dose (%)	0 (Control)	0.2	1.0	5.0
External examination				
Total no. of fetuses (litters) examined	267 (20)	285 (20)	293 (20)	270 (20)
Total no. of fetuses (litters) with abnormalities	1 (1)	1 (1)	0	0
Spina bifida	1 (1)	0	0	0
Thread-like tail and anal atresia	0	1 (1)	0	0
Skeletal examination				
Total no. of fetuses (litters) examined	139 (20)	147 (20)	152 (20)	140 (20)
Total no. of fetuses (litters) with abnormalities	0	0	1 (1)	0
Supernumerary of thoracic vertebral bodies and malpositioned thoracic vertebrae	0	0	1 (1)	0
Total no. of fetuses (litters) with variations	28 (11)	23 (12)	52 (17)	69 (20)**
Bipartite ossification of thoracic centra	1 (1)	1 (1)	1 (1)	7 (5)
Dumbbell ossification of thoracic centra	0	1 (1)	2 (2)	3 (3)
Bipartite ossification of lumbar centra	0	0	0	2 (2)
Supernumerary lumbar vertebrae	4 (1)	1 (1)	5 (3)	5 (2)
Lumbarization	0	0	1 (1)	1 (1)
Bipartite ossification of sternebrae	1 (1)	1 (1)	1 (1)	1 (1)
Misaligned sternebrae	0	0	0	1 (1)
Cervical ribs	2 (2)	3 (3)	3 (3)	7 (5)
Full supernumerary ribs	5 (2)	1 (1)	10 (5)	9 (5)
Short supernumerary ribs	20 (10)	18 (9)	43 (16)	55 (19)**
Wavy ribs	0	0	0	1 (1)
Degree of ossification[a]				
No. of sacral and caudal vertebrae	8.4 ± 0.5	8.4 ± 0.4	8.3 ± 0.5	8.1 ± 0.3
No. of sternebrae	5.9 ± 0.2	5.8 ± 0.2	5.8 ± 0.2	5.8 ± 0.2
No. of forepaw proximal phalanges	1.6 ± 1.3	1.6 ± 0.9	1.2 ± 1.1	0.3 ± 0.4**
Internal examination				
Total no. of fetuses (litters) examined	128 (20)	138 (20)	141 (20)	130 (20)
Total no. of fetuses (litters) with abnormalities	7 (6)	9 (6)	11 (8)	14 (9)
Membranous ventricular septum defect	1 (1)	1 (1)	0	3 (3)
Double aorta	1(1)	0	0	0
Left umbilical artery	1 (1)	0	1 (1)	0
Thymic remnant in neck	4 (4)	8 (5)	10 (8)	11 (8)

** Significantly different from the control, $P < 0.01$.
[a] Values are given as the mean ± SD.

and/or internal abnormalities were found in all groups. The abnormalities observed in the present study are not thought to be due to the administration of 1-butanol, because they have occurred at a very low incidence and are of types that occur sporadically among control rat fetuses (Kameyama et al., 1980; Morita et al., 1987; Nakatsuka et al., 1997; Barnett et al., 2000). Several types of skeletal variations were also found in the control and groups treated with 1-butanol. These skeletal variations are frequently observed in fetuses of rats at term (Kimmel and Wilson, 1973; Kameyama et al., 1980; Morita et al., 1987; Nakatsuka et al., 1997; Barnett et al., 2000). In the 5.0% group, a significant increase in the incidence of fetuses with skeletal variations and fetuses with short supernumerary ribs, but not full supernumerary ribs, and a significant decrease in the degree of ossification were accompanied by a significant decrease in the fetal weight. These findings show a correlation between these morphological alterations and growth retardation in fetuses. Although a skeletal variation, i.e., full supernumerary ribs, is a warning sign of possible teratogenicity, short supernumerary ribs, sternebral variations, and bilobed centra of the vertebral column are normal variations (Kimmel and Wilson, 1973). Chahoud et al. (1999) noted that variations are unlikely to adversely affect survival or health and this might result from a delay in growth or morphogenesis that has otherwise followed a normal pattern of development. Consideration of these findings together suggests that the morphological changes in fetuses observed in the present study do not indicate a teratogenic response and that 1-butanol possesses no teratogenic potential in rats.

In Sitarek's study (1994), significant increases in the incidences of wavy ribs at 300 mg/kg/day, dilation of the subarachnoid space and dilation of the lateral ventricle and/or third ventricle of the brain at 300 mg/kg/day and higher, dilation of the renal pelvis and external hydrocephaly at 1000 mg/kg/day, internal hydrocephaly at 1000 mg/kg/day and higher, and supernumerary ribs and delayed ossification at 5000 mg/kg/day were found. A significant decrease in fetal crown-rump length was

also observed at 5000 mg/kg/day. Based on these findings, Sitarek et al. (1994) concluded that 1-butanol had adverse effects on the morphological development of fetuses in rats. However, we did not confirm their findings. We have demonstrated here that prenatal 1-butanol has no adverse effect on the morphological development of rat offspring. There are some differences between Sitarek's study and the present study in experimental conditions, such as duration of administration and rat strain used in the experiments. Sitarek et al. (1994) administered 1-butanol to female rats for 8 weeks before mating and throughout the mating and pregnancy period and found fetal anomalies, such as hydrocephaly and dilation of the cerebral ventricles and the renal pelvis. On the other hand, we gave 1-butanol to female rats during the whole period of pregnancy and did not detect fetuses with these anomalies. Administration during the premating and mating period is thought to be excluded from the susceptible period for induction of morphological anomalies such as hydrocephaly/dilation of the cerebral ventricles and dilation of the renal pelvis, because rat fetuses are susceptible to induction of these anomalies during mid and late pregnancy (Wood and Hoar, 1972; Kameyama, 1985). The strain difference of rats used in the experiments may explain the discrepancy in the findings regarding fetal anomalies between the studies. In Sitarek's study (1994), ImP: DAK rats obtained from their own breeding colony were used. No detailed information on this strain of rats was available (Sitarek et al., 1994). In their study, dilation of the lateral ventricle and/or third ventricle of the brain was observed in 2% of fetuses (one of the 12 litters) in the control group. In their another study using Imp: DAK rats, extension of the lateral ventricle and/or third ventricle of the brain was observed in 11.7% of fetuses (8 of the 17 litters) in the control group (Sitarek et al., 1996). However, these anomalies were not found in the control group of their studies using Wistar rats (Baranski et al., 1982), Imp: Lodz rats (Sitarek, 1999, 2001) and Imp: WIST rats (Sitarek and Sapota, 2003). The incidences of dilation of the cerebral ventricles in Imp: DAK rats are thought to be higher than those in the background control data of other strains of rats. The fetal incidence of hydrocephaly/dilation of cerebral ventricles in the control rats of reproductive studies conducted between 1986 and 1993 in 63 research institutes is reported to be 0–0.09% and 0–0.26%, respectively (Nakatsuka et al., 1997). In Crj: CD (SD) IGS rats which were used in the present study, the incidence of dilation of the lateral ventricles of the brain in 19 studies conducted during 1998–2000 is reported to be 0–0.06% in fetuses and 0–0.44% in litters (Barnett et al., 2000). Thus, hydrocephaly/dilation of the cerebral ventricle is not commonly observed in fetuses of common strains of rats.

The difference in terminology used for classification of structural anomalies in fetuses may also explain the discrepancy in the findings regarding fetal anomalies between the studies. Sitarek et al. (1996) stated that minor abnormalities, such as enlarged lateral ventricle and/or third ventricle, are quite frequent in rat fetuses and without having the dose-dependent relationship should not be taken alone as evidence of tested chemical fetotoxicity. However, the Fourth Berlin Workshop on Terminology in Developmental Toxicity noted that changes affecting brain ventricles are more likely to be classified as malformations and classification should be based on the historical control incidences, the nature of the organ affected and the severity (Solecki et al., 2003). In Sitarek's study (1994), dilation of the subarachnoid space was observed in fetuses of rats given 1-butanol at 300 mg/kg/day and higher. This anomaly was also found in fetuses in Imp: DAK rats given *N*-cyclohexyl-2-benzothiazolesulfenamide (Sitarek et al., 1996) and Imp: Lodz rats given *N*-methylmorpholine (Sitarek, 1999). No information on the definition of this anomaly was available in their reports. We are unaware of this anomaly in other literature (Kameyama et al., 1980; Morita et al., 1987; Nakatsuka et al., 1997; Horimoto et al., 1998; Barnett et al., 2000; Solecki et al., 2003).

In conclusion, the administration of 1-butanol to pregnant rats throughout pregnancy had adverse effects on maternal rats and embryonic/fetal growth but had no adverse effects on fetal morphological development even at a maternally toxic dose. The data indicate that 1-butanol induces developmental toxicity only at maternally toxic doses in rats. Based on the significant decreases in maternal body weight gain and fetal weight at 5.0%, it is concluded that the NOAELs of 1-butanol for both dams and fetuses are 1454 mg/kg/day (1.0% in drinking water) in rats.

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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 leisions 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					TDI or VSD* (mg/kg/day)	allocation (%)	body weight (kg)	water consump. (L)	guideline value (mg/L)
		inter-species	intra-species	use of LOAEL	study period	nature of toxicity					
DEHP(WHO)[a]											
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

[a]: Derived from the 2nd edition.

¶: At the initial calculation from experimental animal data, the guideline concentration of 0.0005 mg/L was derived as 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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OECD化学物質対策の動向（第5報）
第12回及び第13回OECD高生産量化学物質初期評価会議（2001年）

高橋美加・平田睦子・松本真理子・広瀬明彦・鎌田栄一・長谷川隆一・江馬　眞#

Progress on OECD Chemicals Programme

Mika Takahashi, Mutsuko Hirata, Mariko Matsumoto, Akihiko Hirose, Eiichi Kamata, Ryuichi Hasegawa and Makoto Ema #

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The twelfth SIDS, the Screening Information Data Set, Initial Assessment Meeting (SIAM 12) was held at the Organisation for Economic Co-operation and Development (OECD) headquarters in Paris, France and SIAM 13 was held in Bern, Switzerland, hosted by the European Commission. Two substances at SIAM 12 (CAS No:91-15-6, 123-77-3) and 4 substances at SIAM 13 (CAS No:91-76-9, 112-85-6, 868-77-9, 1477-55-0)were submitted by the Japanese Government and/or International Council of Chemical Associations (ICCA). These substances were agreed at the meetings. In this report, the human health effects of 6 substances above-mentioned are introduced.

Keywords: OECD, HPV program, SIAM, SIDS Initial Assessment Meeting

はじめに

経済協力開発機構（Organization for Economic Cooperation and Development：OECD）加盟各国における高生産量化学物質（High Production Volume Chemical：HPV）の安全性は，1992年に始まったOECD高生産量化学物質点検プログラム（HPV program）によって評価されている[1]．加盟各国での生産量・既存の毒性データ量に基づいてOECD HPV Chemicals Listの作成及び評価の優先順位付けが行われた[1]．現在は，加盟各国と企業が，生産した化学物質に関する情報収集や試験を行って評価文書を完成させ，順次，それらの文書が初期評価会議（SIAM：SIDS, Screening Information Data Set, Initial Assessment Meeting）で討議されている．日本政府は初回より評価文書を提出しており，第6回までに27物質の評価文書について合意を得た．第7回から第11回のSIAMにおいて日本政府が担当し，結論及び勧告が合意された化学物質の初期評価文書の健康影響部分については既に紹介された[2)3)4)]．

SIAMで評価された物質数は2000年までは年間20程度（最多31，最少8）であったが，SIAM 11（2001年）から始まったICCA（International Council of Chemical Associations，国際化学工業協会協議会）による評価文書の提出に伴い，2001年には年間79（SIAM 11-13）と飛躍的に評価物質が増加した．

本稿では，SIAM 12及び13で合意に至った化学物質名と日本担当物質の初期評価要旨の健康影響部分について紹介する．

SIAMにおける合意はFWまたはLPとして示される（FW = The substance is a candidate for further work. LP = The substance is currently of low priority for further work.）．FWは「今後も追加の調査研究作業が必要である」ということを意味する．LPは「現状の使用状況においては追加作業の必要はない」ということを意味し，状況によっては追加作業が必要となる可能性を含む．現在，SIAMでFWとされたのは約100物質，LPは約300物質である．

SIAM 12及び13で合意された化学物質名と日本担当物質の初期評価内容

SIAM 12は2001年6月にフランス（パリ）で開催され，化学物質の初期評価文書が検討され，表1に示す14物質の初期評価結果及び勧告が合意された．SIAM 13は2001年11月にスイス（ベルン）で開催され，化学物質の初期評価文書が検討され，表2に示す36物質の初期評価結果及び勧告が合意された．日本政府が担当した化学物質の初期評価報告書のヒトへの健康影響について，概要を以下に示す．

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Table 1. Chemical substances discussed at SIAM12 and their outcomes

CAS No.	Name of substance	Sponsor country	Outcome
75-68-3	1-Chloro-1,1-difluoroethane	FR/ICCA	LP
79-06-1	Acrylamide	UK:eu	FW
84-74-2	Dibutyl phthalate	NL:eu	FW
91-15-6	*o*-Phthalodinitrile	JP+DE/ICCA	LP
100-21-0	Terephthalic acid	US+IT	LP
105-60-2	Epsilon-Caprolactam	DE/ICCA	LP
123-77-3	Azodicarboxamide	DE+JP	LP
126-73-8	Tributyl phosphate	US	LP
141-97-9	Ethyl acetoacetate	DE:eu	LP
822-06-0	1,6-Hexamethylene diisocyanate	DE/ICCA	FW
1717-00-6	1,1-Dichloro-1-fluoroethane	US/ICCA	LP
25154-52-3	Nonyl phenol	UK:eu	FW
34590-94-8	Dipropylene glycol methyl ether	US/ICCA	LP
84852-15-3	Phenol, 4-nonyl-, branched	UK:eu	FW

Note. Abbreviations show DE: Germany, FR: France, IT: Italy, JP: Japan, NL: Netherlands, UK: United Kingdom, and US: United States of America. ":eu" indicates the document was based on the risk assesment in European Communities.

Table 2. Chemical substances discussed at SIAM13 and their outcomes

CAS No.	Name of substance	Sponsor country	Outcome
58-55-9	Theophylline	DE/ICCA	LP
65-85-0	Benzoic acid	NL/ICCA	LP
68-12-2	N,N-Dimethylformamide	DE/ICCA	FW
71-36-3	*n*-Butyl alcohol	US/ICCA	LP
74-83-9	Methyl bromide	US/ICCA	LP
75-01-4	Vinyl chloride	US/ICCA	LP
75-38-7	Vinylidene fluoride	US/ICCA	LP
75-56-9	Methyl oxirane	UK:eu	FW
79-10-7	Acrylic acid	DE:eu	FW
79-20-9	Methyl acetate	DE:eu	FW
88-73-3	1-Chloro-2-nitrobenzene	DE/ICCA	FW
88-74-4	2-Nitroaniline	FR/ICCA	LP
91-76-9	2,4-Diamino-6-phenyl-1,3,5-triazine	JP/ICCA	LP
95-50-1	1,2-Dichlorobenzene	Aus	FW
100-51-6	Benzyl alcohol	NL/ICCA	LP
103-84-4	Acetanilide	KO	LP
107-15-3	Ethylenediamine	US/ICCA	LP
107-41-5	Hexylene glycol	UK/ICCA	LP
108-77-0	Cyanuric chloride	CH/ICCA	LP
109-66-0	*n*-Pentane NO:eu		LP
112-57-2	Tetraethylenepentamine	US/ICCA	LP
112-85-6	Docosanoic acid	JP/ICCA	LP
123-54-6	2,4-Pentanedione	DE/ICCA	FW
123-86-4	*n*-Butyl acetate	US/ICCA	LP
127-19-5	N,N-Dimethylacetamide	IT	LP
532-32-1	Sodium benzoate	NL/ICCA	LP
582-25-2	Potassium benzoate	NL/ICCA	LP
616-38-6	Dimethyl carbonate	IT/ICCA	Not finalized
868-77-9	2-Hydroxyethyl methacrylate	JP/ICCA	LP
1310-58-3	Potassium hydroxide	BE/ICCA	LP
1477-55-0	1,3-Bis(aminomethyl)benzene	JP/ICCA	LP
5392-40-5	Citral	JP	LP
6386-38-5	Metilox	CH	LP
6864-37-5	2,2'-Dimethyl-4,4'-methylenebis (cyclohexylamine)	DE/ICCA	LP
7447-40-7	Potassium chloride	NO/ICCA	LP
7681-57-4	Disodium disulphite	KO/ICCA	LP
16470-24-9	Fluorescent Brightener 220	DE/ICCA	FW

Note. Additional abbreviations to table 1, Aus: Australia, CH: Switzerland, NO: Norway, KO: Korea, and BE: Belgium. Health effects of citral have already described in SIAM11.

o-Phthalodinitrile (91-15-6) (ICCA 日本及びドイツ企業作成) (SIAM 12)

本化学物質はフタロシアニン系染料, 顔料の原料として用いられている.

単回経口投与毒性試験 (OECD TG 401) では, ラットの雌雄ともに 60 mg/kg 以上の投与で死亡, 痙攣, 口周囲の汚れがみられ, 240 mg/kg以上の投与で自発運動低下, 腹臥位, チアノーゼ等が観察された. 経口LD_{50}は85 mg/kgであった.

吸入毒性試験では, 20℃で 8 時間の飽和蒸気圧に暴露させたラットの死亡はみられなかった.

皮膚及び眼に対する刺激性はみられなかった. 皮膚感作性に関する情報はなかった.

反復投与毒性・生殖発生毒性併合試験 (OECD TG 422) では, 0, 1, 6, 30 mg/kg/day を雌雄のラットに少なくとも 42 日間強制経口投与した. 30 mg/kg/day の雌雄で体重増加抑制及び摂餌量の減少, 雄では総コレステロール及び総蛋白の増加, 血清尿窒素の減少, 肝臓, 腎臓及び精巣重量の増加, 精巣上体重量の減少, 雌では全例が妊娠末期に痙攣を伴い死亡した. また, 30 mg/kg/dayの雄において肝臓の小葉中心性肝細胞肥大, 腎臓の近位尿細管上皮における硝子滴沈着, 精細管萎縮及び精巣上体の管腔内の細胞残屑出現と精子数の減少が観察された. 6 mg/kg/day 投与では雌雄とも毒性所見はみられなかったので, この試験における反復投与毒性の無毒性量 (NOAEL) は 6 mg/kg/day であった.

90 日間反復経口投与毒性試験 (OECD TG 408) 及び米国 EPA 神経毒性試験ガイドラインに従い, 雄ラットに 0, 3, 8, 25 mg/kg/day, 雌ラットに 0, 3, 10, 30 mg/kg/day の用量を 13 週間混餌投与したところ, 自発運動量の増加がみられたが, 中枢及び末梢神経毒性に関連した症状及び神経病理学的変化は観察されなかった. 雄は 25 mg/kg/day, 雌は 10 mg/kg/day で体重減少がみられたことから, この試験における反復経口投与の NOAEL は雄ラットでは 8 mg/kg/day, 雌ラットでは 3 mg/kg/day であった.

これらの結果から, 反復経口投与毒性の NOAEL は 3 mg/kg/day と考えられた.

上述の反復投与毒性・生殖発生毒性併合試験において, 雄ラットには交配前後の 14 日間ずつを含む少なくとも 42 日間, 雌ラットには交配前 14 日間から哺育 3 日まで, 0, 1, 6, 30 mg/kg/dayを強制経口投与した. 30 mg/kg/dayにおいて, 雄で精巣毒性がみられ, さらに, すべての妊娠ラットが死亡したため分娩児のデータは得られなかった. 6 mg/kg/day 以下の用量では生殖発生に対する影響がみられなかったことから, 生殖発生毒性のNOAELは 6 mg/kg/dayと考えられた.

細菌を用いた復帰突然変異試験の結果は S9mix 存在下及び非存在下のいずれでも陰性であった. チャイニーズ・ハムスター培養細胞を用いた染色体異常試験では, いずれの連続処理でも染色体の構造異常は認められなかったが, 中濃度 (0.40 mg/mL) 及び高濃度において倍数体が誘発された. また, S9mix 存在下及び非存在下のいずれの短時間処理でも染色体の構造異常は認められなかったが, S9mix 存在下及び非存在下のいずれの短時間処理でも倍数体が誘発され, これらの結果より陽性と判断された. しかしながら, *in vivo* でのマウスの小核試験で投与可能な最高投与量20 mg/kg の結果が陰性であったことから, 本化学物質は *in vivo* では遺伝毒性を発現しないと考えられた.

発がん性に関する有効な情報はなかった.

Azodicarboxamide（123-77-3)（ドイツ政府及び日本政府作成）(SIAM 12)

本化学物質は，プラスティックやゴム製品の発泡剤，米国の食品添加物（膨張剤）として用いられている．

雄ラットへの吸入暴露で本化学物質の約34%が，経口投与で10-33%が吸収されるが，本化学物質のかなりの量は胃腸管で吸収されず，糞とともに排泄される．本化学物質は吸収後速やかにbiurea（CAS 110-21-4)に代謝物され，72時間以内に主に尿中に排泄される．

急性経口毒性は弱く，雄ラットでの2つの試験では2,500 mg/kgで毒性発現はみられず，別の試験ではLD_{50}は4,000 mg/kg以上（雌雄Alderly Parkラット），5,000 mg/kg以上（雄Wistarラット）であった．

雌雄ラット（1群5匹）を用いた急性吸入毒性試験では，LC_{50}は6,100 mg/m³以上（4時間暴露，粒子サイズ5.8 μm）であった．4時間暴露後，6,100 mg/m³で10例中8例が呼吸困難を示したが，死亡はみられなかった．暴露後14日での病理組織検査では影響はみられなかった．

モルモットでの吸入刺激試験では，肺機能に影響はみられず，97 mg/m³（1時間）までの濃度において吸入刺激は極軽微であった．

急性皮膚毒性試験が5匹の雄ラットに500 mg/kgを塗布して行われ，毒性徴候や死亡はみられなかった．
1匹の雌ウサギに2,000 mg/kgを塗布したスクリーニング試験でも毒性徴候はみられなかった．

ウサギの皮膚への刺激性はみられず，眼に対しては可逆的な角膜の発赤や腫脹がみられた．ヒトの皮膚を用いたパッチテストが陽性であったことから，皮膚感作性の可能性が示された．

ラットに100, 500, 2,500 mg/kg/day（雄），200, 1,000, 5,000 mg/kg/day（雌）を強制経口投与した90日間反復経口投与毒性試験では，雄の2,500 mg/kg/dayと雌の5,000 mg/kg/day（雌）で死亡がみられたが，一般毒性の徴候はみられず，病理組織学的検査で腎盂腎炎等がみられた．NOAELは500 mg/kg/day（雄），1,000 mg/kg/day（雌）であった．

また，マウスに0, 78, 156, 312, 625, 1,250 mg/kg/day（雄），0, 156, 312, 625, 1,250, 2,500 mg/kg/day（雌）を強制経口投与した90日間反復経口投与毒性試験では，最高用量でも投与に関連した影響はみられなかった．

一世代生殖毒性試験（OECD TG 415）では，ラットに0, 100, 300, 1,000 mg/kg/dayの用量で強制経口投与した．雄親ラットには最高用量でも影響はみられなかったが，雌親ラットでは1,000 mg/kg/dayで腎盂の拡張，間質のリンパ球浸潤等の腎臓への影響がみられた．NOAELは300 mg/kg/dayであった．

これらの試験の結果から，反復経口投与のNOAELは500 mg/kg/day（雄），300 mg/kg/day（雌）とされた．

13週間吸入試験において，ラット及びマウスに0, 50, 10, 200 mg/m³の濃度を1日6時間（週5日）で暴露させたところ，最高濃度の200 mg/m³でも有意な毒性影響はみられなかった．13週間暴露試験に基づき，吸入反復暴露のNOAELは200 mg/m³（ラット，マウス）であった．

上述の一世代生殖毒性試験では，雄ラットには98日間，雌ラットには交配前14日間から哺育20日まで，それぞれ0, 100, 300, 1,000 mg/kg/dayを強制経口投与した．最高投与量の1,000 mg/kg/dayでも生殖発生への毒性影響はみられず，生殖発生毒性のNOAELは1,000 mg/kg/dayと考えられた．ラットとマウスを200 mg/m³の濃度まで暴露した上述の13週間吸入試験においても生殖器系や精子の形態，発情周期に影響は認められなかった．

細菌を用いた復帰突然変異試験の結果はS9mix存在下及び非存在下のいずれでも陰性であった．チャイニーズ・ハムスター培養細胞を用いた染色体異常試験では，いずれの連続処理及びS9mix存在下及び非存在下のいずれの短時間処理でも染色体の構造異常及び倍数体の誘発は観察されなかったことから，染色体異常試験は陰性と判断された．

発がん性に関する有効な情報はなかった．

Docosanoic acid（112-85-6)（ICCA日本企業作成）(SIAM 13)

本化学物質は近年化粧品の原料として用いられている．

トキシコキネティクスに関する情報はなかった．

単回経口投与毒性試験（OECD TG 401）では，最高投与量の2,000 mg/kgでもラットの死亡はみられず，また，一般状態，投与後の体重の推移及び剖検所見のいずれにも投与に起因すると考えられる変化は観察されず，経口LD_{50}は2000 mg/kg以上であった．

皮膚及び眼に対する刺激性，皮膚感作性に関する情報はなかった．

反復投与毒性・生殖発生毒性併合試験（OECD TG 422）では，雄ラットには42日間，雌ラットには交配前14日間から哺育3日まで，それぞれ0, 100, 300, 1,000 mg/kg/dayを強制経口投与した．雄ではいずれの投与群においても死亡及び一般状態の異常は観察されなかった．また，42日間反復投与後の剖検，病理組織学的検査，血液学検査及び血液生化学検査でも，投与の影響を示唆する所見または異常値は認められなかった．雌でもいずれの投与群においても死亡及び一般状態の異常は観察されなかった．分娩後4日の剖検及び病理組織学的検査においても投与の影響を示唆する所見は認められなかった．反復経口投与のNOAELは1,000 mg/kg/dayと考えられた．

上述の反復投与毒性・生殖発生毒性併合試験において，最高投与量の1,000 mg/kg/dayでも交尾能及び受胎率に影響はみられなかった．また，母動物の妊娠期間，出産率，分娩状態及び哺育状態に投与の影響を示唆する変化は認められなかった．出生児の性比，体重及び生存率に，投与の影響を示唆する変化は認められなかった．また，出生児の形態異常は

いずれの投与群にも観察されなかった．生殖発生毒性のNOAELは1,000 mg/kg/dayと考えられた．

細菌を用いた復帰突然変異試験の結果はS9mix存在下及び非存在下のいずれでも陰性であった．チャイニーズ・ハムスター培養細胞を用いた染色体異常試験では，いずれの連続処理及びS9mix存在下及び非存在下のいずれの短時間処理でも染色体の構造異常及び倍数体の誘発傾向は観察されなかったので，染色体異常試験は陰性と判断された．

発がん性に関する情報はなかった．

2,4-Diamino-6-phenyl-1,3,5-triazine (91-76-9) (ICCA日本企業作成) (SIAM 13)

本化学物質はベンゾグアナミンーホルムアルデヒドの中間体として使用される．

トキシコキネティクスに関する情報はなかった．

単回経口投与毒性試験（OECD TG 401）では0, 250, 500, 1,000, 2,000 mg/kgを投与したところ，雌雄とも1,000 mg/kg以上の投与で死亡がみられた．死亡例では，前胃で肉眼的に粘膜の肥厚，病理組織学的に粘膜下組織の浮腫がみられ，脾臓及び胸腺では肉眼的及び病理組織学的に萎縮がみられた．また，膀胱では濃緑色尿の貯留がみられた．生存例では，前胃に肉眼的に粘膜の白色点がみられ，病理組織学的には粘膜に扁平上皮の過形成がみられた．これらより，ラットでの経口LD_{50}は雄で933 mg/kg，雌で1,231 mg/kgであった．

吸入毒性試験（OECD TG 403）では，ラットでの吸入LC_{50}は2.932mg/L（4時間）であった．

ウサギにおいて，皮膚への刺激性はなく，眼への刺激性は軽度であった．皮膚感作性に関する情報はなかった．

反復投与毒性・生殖発生毒性併合試験（OECD TG 422）では，雌雄ラットに少なくとも39日間，それぞれ0, 4, 20, 100 mg/kg/dayを強制経口投与した．雌雄の100 mg/kg/dayで1例ずつの死亡がみられた．雄の血液学検査では，100 mg/kg/dayの赤血球数及びヘマトクリット値の減少及び網状赤血球率の増加が認められた．雄の血液生化学検査では，100 mg/kg/dayでアルブミン，A/G比，GOT，GPT，総コレステロール，リン脂質及び総ビリルビンの増加ならびにトリグリセライド，ナトリウム及びカリウムの減少が認められた．100 mg/kg/dayの雄で肝臓重量の増加がみられ，病理組織学的には雌雄で小葉中心性肝細胞肥大が認められた．また，死亡例では，100 mg/kg/dayの雄1例で回腸の粘膜固有層から漿膜にかけて好中球性の細胞浸潤及び肉芽形成がみられたほか，腺胃のびらん，肺の水腫，脾臓の萎縮，胸腺の萎縮及び出血がみられ，同群の雌1例で腺胃のびらん，肺の水腫，脾臓の萎縮及び副腎の壊死が認められた．これらに結果から，ラットにおける反復経口投与のNOAELは20 mg/kg/dayと考えられた．

90日間反復経口投与毒性試験（OECD TG 408）に従い，雄ラットに90日間0, 1.9, 19.0, 173.0 mg/kg/dayを混餌投与した反復投与毒性試験では，死亡はみられず，173.0 mg/kg/dayにおいて，雌雄ラットで弓なり姿勢，立毛，体重増加量の減少，摂餌量の減少がみられた．血液化学では，雌雄ラットでGPT及びビリルビンが増加し，雌の肝臓重量が増加した．病理組織学的検査では，小葉中心性肝細胞肥大，脾髄外造血の亢進，副腎球状帯細胞の異常発達及び空胞形成，炎症細胞の湿潤を伴う膵臓外分泌細胞の退化が観察された．また，ヘモジデリン色素沈着の増加が雌雄ラットの腎臓及び脾臓で観察された．19 mg/kg/dayの雄では脾臓のヘモジデリン色素沈着の増加が投与に関連した唯一の病理組織学的変化として認められたが，この変化は穏やかであり，他の影響は観察されなかった．これらの結果にもとづき，混餌による反復経口投与のNOAELは19 mg/kg/dayと考えられた．

反復投与毒性・生殖発生毒性併合試験（OECD TG 422）では，雄ラットには交配の14日前から49日間，雌ラットには交配前14日間から哺育3日まで，それぞれ0, 4, 20, 100 mg/kg/dayを強制経口投与した．親動物の生殖機能に関しては，性周期，黄体数，交尾率，着床痕数，授（受）胎率及び交尾所要日数に投与の影響は認められなかった．分娩時の検査では，100 mg/kg/dayの2例で分娩直後の児の回集及び保温の不良などが認められた．さらに，100 mg/kg/dayで死産率の増加及びそれに伴う出生率の減少，雌雄新生児体重の減少が認められた．各群とも妊娠期間，出産児数，出産率，新生児数及び新生児の性比では投与の影響はみられず，新生児の外表検査においても，異常は認められなかった．哺育期の検査では，20 mg/kg/dayの2例及び100 mg/kg/dayの7例で児の回集，授乳，保温などの哺育行動の不良がみられ，これらの母動物では全児が死亡した．また，20 mg/kg/day以上では母動物の哺育行動の不良に起因した新生児の4日の生存率の減少が認められた．さらに，100 mg/kg/dayでは新生児の哺育4日の体重に低値が認められた．これらより，雄では100 mg/kg/dayで影響がみられず，雌では20 mg/kg/dayで哺育行動の異常がみられた．最高用量でも児に形態異常はみられなかった．これらの結果から，生殖毒性のNOAELは100 mg/kg/day（雄），4 mg/kg/day（雌）であった．また，100 mg/kg/dayで児の体重減少がみられたので，発生毒性のNOAELは20 mg/kg/dayであった．

細菌を用いた復帰突然変異試験の結果はS9mix存在下及び非存在下のいずれでも陰性であった．チャイニーズ・ハムスター培養細胞を用いた染色体異常試験では，連続処理法の48時間処理及びS9mix存在下の短時間処理で染色体構造異常の誘発作用が認められた．また，連続処理法の48時間処理による試験では，倍数体の誘発作用が認められた．連続処理法の24時間処理による試験では，用量に依存した染色体構造異常の誘発作用が認められた．ヒト・リンパ培養細胞を用いた染色体異常試験では，S9mix存在下及び非存在下のいずれでも本化学物質の溶解限度内では染色体構造異常の誘発作用は認められず，S9mix非存在下で溶解限度を超え